

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: NDA 21174

PHARMACOLOGY REVIEW(S)

SEP 12 1996

Division of Oncology Drug Products

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA Review #2

IND: []
Serial # 029

Submission: IND Dated: 8/15/96
Received by CDER: 8/19/96

Information to be conveyed to the sponsor: Yes (X) No ()

Reviewer: Paul A. Andrews, Ph.D.

Date Review Completed: 9/5/96

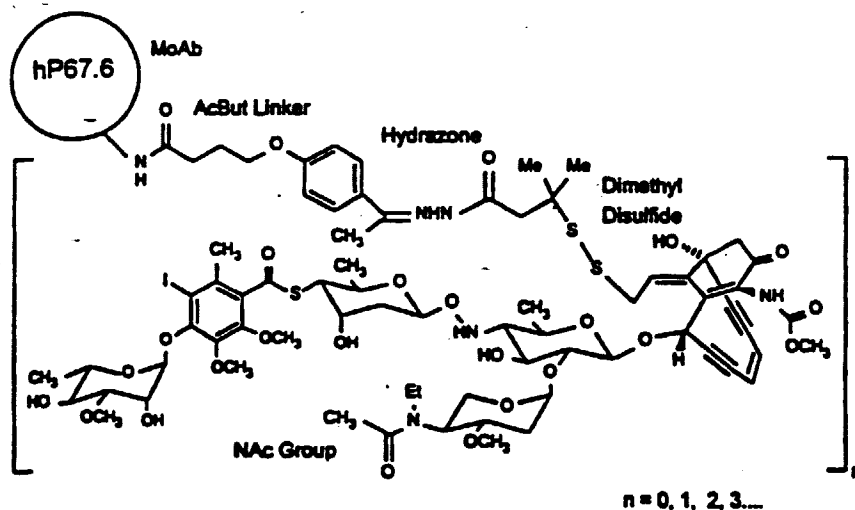
Sponsor: Wyeth-Ayerst Research
Philadelphia, PA

Drug Name: Primary: CMA-676
Secondary: hP67.6 conjugate; 555,201;
hP67.6-NAc- γ_1^1 -calicheamicin DMH AcBut conjugate

Other Relevant Names: 184,538 NAc- γ -calicheamicin DMH
191,305 NAc- γ -calicheamicin DMH AcBut
181,441 γ_1^1 -calicheamicin
555,001 hP67.6 "naked" antibody
181,287 ϵ -calicheamicin ("triggered form")
190,396 NAc- ϵ -calicheamicin

CAS number: not known

Structure: The MoAb is a humanized murine monoclonal antibody to human CD33.



Molecular weight and formula: not known

Related INDs, NDAs: IND []

Pharmacologic Class: Antineoplastic

Indication: leukemia

Clinical Formulation:

Format	Ingredient	Amount
freeze-dried powder	CMA-676	2.5 mg protein/vial reconstituted to 1 mg protein/ml

Route of administration and dosage form: dose administered in 100 ml of saline containing 13% HSA as a 2 hr i.v. infusion

Ongoing Clinical Study:

Previous Review(s), Date(s), and Reviewer(s): IND, 12/29/94, Paul A. Andrews, Ph.D.

Studies Reviewed in This Submission_____

V. Special Toxicity

28070 Mutagenicity test on CMA-676/calicheamicin in an *in vivo* mouse micronucleus test (SE #029, p. 1-45)

Studies Previously Reviewed_____

I. Pharmacology

22. Anti-CD33-N-Ac gamma calicheamicin inhibits colony forming activity from blood and bone marrow of diagnostic AML patient samples. (Vol 1.9, p. 95-107)
23. Binding characteristics of hP67.6 antibody and hP67.6 conjugate to normal human peripheral blood leukocytes and bone marrow cells: comparative flow cytometric analysis. (Vol 1.9, p. 108-147)
26. Evaluation of relative immunoaffinities of four batches of hP67.6-N-acetyl γ^1 calicheamicin dimethyl AcBut (CL 555,201). (Vol 1.9, p. 175-179)
27. Evaluation of hydrazide-, amide-, and "hybrid"-linked conjugates of N-acetyl γ^1 calicheamicin (CL181,927) with murine and humanized versions of the anti-CD33 antibody, P67.6, against the HL-60 tumor as a model of acute myeloid leukemia (AML). (Vol 1.9, p. 180-213)
55. In vitro cellular cytotoxicity of NAc-calicheamicin gamma (CL181,927), NAc-calicheamicin gamma DMH (CL184,538), NAc-calicheamicin gamma DMH AcBut Acid (CL191,305), and hP67.6-NAc-calicheamicin gamma DMH AcBut conjugate (CL 555,201). (Vol 1.11, p. 71-77)
44. Synthesis and in vitro cellular cytotoxicity of epsilon calicheamicin (CL 181,287) and N-acetyl epsilon calicheamicin (CL 190,396). (Vol 1.10, p. 93-100)

II. Safety Pharmacology

24. Reactivity of humanized hP67.6 conjugate (CPP771/CL555201) to a panel of normal human tissues. (Vol 1.9, p. 148-168)
48. Reaction of N-Ac calicheamicin DMH (CL 184,538) with reduced glutathione. (Vol 1.10, p. 129-140)
54. Hydrolysis of hP67.6-N-Ac gamma calicheamicin DMH AcBut conjugate (CL 555,201) in buffer at physiologically relevant pH's. (Vol 1.11, p. 59-79)
56. Reactivity of humanized P67 antibody (CDP771) to normal human tissues. (Vol 1.11, p. 78-96)
18. Reactivity of humanized antibody (CDP771/CL555001) to a panel of normal human tissues. (Vol 1.9, p. 57-77)

57. Cross reactivity of humanized monoclonal antibody hP67.6 with tissues of cynomolgus and Sprague-dawley rats. (Vol 1.11, p. 97-105)
59. Cardiovascular safety assessment of CL 555,201 in conscious beagle dogs, study 94C. (Vol 1.11, p. 122-191)
66. In vitro blood compatibility of CL 555,201 (N-acetyl-gamma-dimethyl-hydrazide-acetyl- butyryloxy derivative of calicheamicin [CL 191,305] conjugated to hP67.6 [CL 555,001]), an anticancer agent. (Study 94052). (Vol 1.18, p. 93-109)
86. The effects of the derivatives of gamma calicheamicin, N-acetyl gamma calicheamicin (CL 181,927), N-acetyl dimethyl hydrazide (CL 184,538), N-acetyl dimethyl acid (CL 186,760) and N-acetyl epsilon (CL 190,396) on murine bone marrow hematopoietic colony formation in vitro (Study 92166). (Vol 1.32, p. 222-239)
93. In vitro stability of hP67.6 conjugate (CL 555,201) in human, monkey, and rat plasma at 37°C for a period of 6 hours. (Vol 1.33, p. 211-253)

III. Pharmacokinetics and Toxicokinetics

89. Pharmacokinetics of the hP67.6/³H-N-Acetyl-γ-DMH-AcBut calicheamicin conjugate (CL 555,201) following a single intravenous administration in rats. (Study A9442). (Vol 1.33, p. 62-108)
90. Pharmacokinetics of the hP67.6/³H-N-Acetyl-γ-DMH-AcBut calicheamicin conjugate (CL 555,201) following a single intravenous administration in cynomolgus monkeys (Study A9452). (Vol 1.33, p. 109-156)
91. Validation of an ELISA assay for quantitation of total calicheamicin derivatives in rat plasma and cross-validation for such quantitation in monkey plasma. (Study TSD358 and TSD260.2) (Vol 1.33, p. 157-185)
92. Validation of an ELISA assay for quantitation of total antibody (hP67.6) in rat plasma and cross-validation for such quantitation in monkey plasma. (Study TSD358 and TSD 260.2) (Vol 1.33, p. 186-210)

IV. Toxicology

A. Single dose Toxicity

60. A single dose intravenous toxicity study of CL 555,201 (N-acetyl-gamma- dimethyl-hydrazide-acetyl-butyryloxy derivative of calicheamicin [CL 191,305] conjugated to hP67.6 [CL 555,001]), an anticancer agent, in rats (Study 93119). (Vol 1.11, p.192-299)
61. An exploratory single dose intravenous tolerance study of CL 555,201 (N-acetyl-gamma-dimethyl-hydrazide-acetyl-butyryloxy derivative of calicheamicin [CL 191,305] conjugated to hP67.6 [CL 555,001]), an anticancer agent, in rats (Study 94033). (Vol 1.12, p. 1-420)
62. A single dose intravenous toxicity study of CL 555,201 (N-acetyl-gamma-dimethyl-hydrazide-acetyl-butyryloxy derivative of calicheamicin [CL 191,305] conjugated to hP67.6 [CL 555,001]), an anticancer agent, in male monkeys (Study 93214). (Vol 1.13, p. 1-60)
65. An exploratory single dose intravenous tolerance study of CL 555,201 (N-acetyl-gamma-dimethyl-hydrazide-acetyl-butyryloxy derivative of calicheamicin [CL 191,305] conjugated to hP67.6 [CL 555,001]), an anticancer agent, in the chimpanzee (Study 94118). (Vol 1.18, p. 1-92)
67. A single intravenous dose exploratory study of calicheamicin (CAL) analogs (antitumor antibiotics) in mice (Study 90126). (Vol 1.18, p. 110-336)
68. A single dose intravenous toxicity study of CL 184,538 (N-acetyl-gamma-dimethyl-hydrazide derivative of calicheamicin), an anticancer agent, in rats (Study 92044). (Vol 1.19, p. 1-427)
69. A single dose intravenous toxicity study of CL 184,538 (N-acetyl-gamma-dimethyl-hydrazide derivative of calicheamicin), an anticancer agent, in dogs (Study 92047). (Vol 1.20, p. 1-414)
72. A single dose intravenous toxicity study of CL 191,305 (N-acetyl-gamma-dimethyl-hydrazide AcBut derivative of calicheamicin), an anticancer agent, in rats (Study 93026). (Vol 1.24, p. 1-445)
73. A single dose intravenous toxicity study of CL 190,396 (N-acetyl-epsilon derivative of calicheamicin), an anticancer agent, in rats (Study 92045). (Vol 1.25, p. 1-394)

B. Multiple dose Toxicity

63. Six Cycle intravenous toxicity study of CL 555,201 (N-acetyl-gamma-dimethyl-hydrazide-acetyl-butyryloxy derivative of calicheamicin [CL 191,305] conjugated to hP67.6 [CL 555,001]), an anticancer agent, in rats followed by a four week recovery period (Study 94034). (Vol 1.13, p. 61 to Vol 1.15 p. 377)
64. A six Cycle intravenous toxicity study of CL 555,201 (N-acetyl-gamma-dimethyl-hydrazide-acetyl-butyryloxy derivative of calicheamicin [CL 191,305] conjugate to hP67.6 [CL 555,001]), an anticancer agent, in monkeys followed by a four week recovery period (Study 94001). (Vol 1.16-1.17)

C. Special Toxicity

8. The use of radiolabeled anti-CD33 antibody to augment marrow irradiation prior to marrow transplantation for acute myelogenous leukemia. Transplantation, 54:829, 1992.(Vol 1.8, p.224-228)

Studies not Reviewed in this Submission—— none

Note that portions of this review were excerpted directly from the sponsor's submission.

V. Special Toxicity**28070 Mutagenicity test on CMA-676/calicheamicin in an *in vivo* mouse micronucleus test.**

Conducted by the [] according to GLP. The method of [] was used. A dose selection study preceded the micronucleus test. At indicated times post-dosing, mice were sacrificed and the bone marrow removed from the femur. The ratio of polychromatic/norchromatic nuclei was calculated and 1000 polychromatic erythrocytes per animal were scored for micronuclei. (Chromatid and chromosome fragments induced by clastogens are left behind in anaphase and included in daughter cells. These form micronuclei in the cytoplasm that persist after the nucleus is extruded by the mature erythrocyte.) The PCE/NCE ratio decreased for at least one time point in all dose levels indicating that cytotoxic plasma levels had been achieved. All dose levels at all time points (except LD σ s at 72 hr) had statistically significant increases in micronuclei. CMA-676 was thus clastogenic in the mouse *in vivo* micronucleus test.

drug: CMA-676 lot# 4489A35-301195-R1592-163
 species: :CD-1 (ICR)BR mice (5/sex/group)
 age; weight: 9 weeks; 26.5-35.3 g for σ and 21.6-29.6 for η
 dosages: 0, 225, 450, and 900 μ g/kg
 route: i.v.
 sampling: 24, 48, and 72 hr
 positive controls: 80 mg/kg cyclophosphamide by oral gavage (24 hr only)

Mean Micronuclei per 1000 PCE (n=5 animals)						
	24 hr		48 hr		72 hr	
	σ	η	σ	η	σ	η
control	0.6	0.0	0.4	1.0	1.0	0.2
225 μ g/kg	11.6	14.2	16.4	29.2	9.8 ^a	17.0
450 μ g/kg	27.6	24.8	30.6	44.7 (4) ^b	42.0	32.8
900 μ g/kg	35.8	39.4	27.3 (4)	37.0 (1)	75.7 (3)	65.3 (3)

^a not statistically significant; ^b number of animals

The Investigator's Brochure should be updated to indicate that CMA-676 is clastogenic *in vivo*.

- a) Comments for further studies: none
b) Discussed with Medical Officer: nothing

CMA-676 is clastogenic *in vivo* (mouse bone marrow micronucleus test).

Draft Letter. Requests for Sponsor

1. Please update the Investigator's Brochure to indicate that CMA-676 is clastogenic *in vivo*.

/s/ ^

Paul A. Andrews, Ph.D.
Pharmacologist

Date 9/5/96

HFD-150

/JJDeGeorge

/JBeitz

PGuinn

/PA Andrews

gpc 9/12/96

Amclirens

FEB 7 1995

Division of Oncology and Pulmonary Drug Products

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA Original, Review #1

IND: []
Serial # 000

Submission: IND Dated: 11/9/94
Received by CDER: 11/10/94

Information to be conveyed to the sponsor: Yes () No ☒

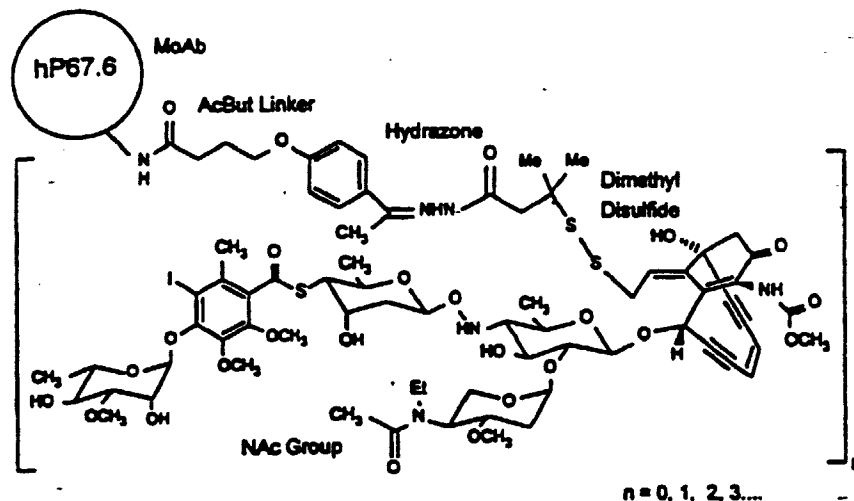
Reviewer: Paul A. Andrews, Ph.D.

Sponsor: Lederle Laboratories
Pearl River, New York

Drug Name: Primary: hP67.6 conjugate
Secondary: 555,201
hP67.6-Nac- γ_1^I -calicheamicin DMH AcBut conjugate

Other Relevant Names: 184,538 NAc- γ -calicheamicin DMH
191,305 NAc- γ -calicheamicin DMH AcBut
181,441 γ_1^I -calicheamicin
555,001 hP67.6 "naked" antibody
181,287 ϵ -calicheamicin ("triggered form")
190,396 NAc- ϵ -calicheamicin

Structure: The MoAb is a humanized murine monoclonal antibody to human CD33.



Related INDs, NDAs: IND []

Pharmacologic Class: Antineoplastic

Indication: leukemia

Clinical Formulation:

<u>Format</u>	<u>Ingredient</u>	<u>Amount</u>
freeze-dried powder	555,201	2.5 mg protein/vial reconstituted to 1 mg protein/ml

Route of administration and dosage form: dose administered in 100 ml of saline containing 13% HSA as a 2 hr i.v. infusion

Proposed Clinical Study: An open-label, single center, single arm dose escalation study in relapsed or refractory CD33⁺ patients to investigate: a) the acute, hematologic, and non-hematologic toxicities and b) the pharmacokinetics of the hP67.6 conjugate in human subjects. Patients will receive up to 3 cycles at least 14 days apart of hP67.6 conjugate as 2 hr infusions. Proposed dosages are 0.25, 0.5, 1, 2, and 4 mg protein/m² (6.25- 100 µg calicheamicin/m² equivalents).

Previous Review(s), Date(s), and Reviewer(s): none

Studies Reviewed in This Submission-----**I. Pharmacology**

22. Anti-CD33-N-Ac gamma calicheamicin inhibits colony forming activity from blood and bone marrow of diagnostic AML patient samples. (Vol 1.9, p. 95-107)
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92. Validation of an ELISA assay for quantitation of total antibody (hP67.6) in rat plasma and cross-validation for such quantitation in monkey plasma. Study TSD358 and TSD 260.2 (Vol 1.33, p. 186-210)

III. Toxicology**A. Single dose Toxicity**

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 67. A single intravenous dose exploratory study of calicheamicin (CAL) analogs (antitumor antibiotics) in mice (Study 90126). (Vol 1.18, p. 110-336)
 68. A single dose intravenous toxicity study of CL 184,538 (N-acetyl-gamma-dimethyl-hydrazide derivative of calicheamicin), an anticancer agent, in rats (Study 92044). (Vol 1.19, p. 1-427)
 69. A single dose intravenous toxicity study of CL 184,538 (N-acetyl-gamma-dimethyl-hydrazide derivative of calicheamicin), an anticancer agent, in dogs (Study 92047). (Vol 1.20, p. 1-414)
 72. A single dose intravenous toxicity study of CL 191,305 (N-acetyl-gamma-dimethyl-hydrazide AcBut derivative of calicheamicin), an anticancer agent, in rats (Study 93026). (Vol 1.24, p. 1-445)
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C. Special Toxicity

8. The use of radiolabeled anti-CD33 antibody to augment marrow irradiation prior to marrow transplantation for acute myelogenous leukemia. Transplantation, 54:829, 1992. (Vol 1.8, p.224-228)
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86. The effects of the derivatives of gamma calicheamicin, N-acetyl gamma calicheamicin (CL 181,927),

N-acetyl dimethyl hydrazide (CL 184,538), N-acetyl dimethyl acid (CL 186,760) and N-acetyl epsilon (CL 190,396) on murine bone marrow hematopoietic colony formation in vitro (Study 92166). (Vol 1.32, p. 222-239)

93. In vitro stability of hP67.6 conjugate (CL 555,201) in human, monkey, and rat plasma at 37°C for a period of 6 hours. (Vol 1.33, p. 211-253)

Studies not Reviewed in this Submission—————

Volume 8

1. Tumor therapy with radiolabeled monoclonal antibodies. *Antibody, Immunoconj. Radiopharm.* 1991; 4:245-27.
2. Approaches to improved antibody- and peptide-mediated targeting for imaging and therapy of cancer. *J. Controlled Release.* 1994; 28:167-173.
3. Immunotoxins against cancer. *Biochim. Biophys. Acta.* 1994; 1198:27-45.
4. Antibody-directed enzyme prodrug therapy (ADEPT). *J. Controlled Release.* 1994; 28:187-193.
5. Current status and future perspectives of chemoimmunoconjugates for the treatment of cancer. In: Borrebaeck CAK, Larrick JW, eds. *Therapeutic Monoclonal Antibodies.* New York: Stockton Press; 1990:57-73.
6. Immunoconjugates containing novel maytansinoids: promising anticancer drugs. *Cancer Res.* 1992; 52:127-131.
7. Immunoconjugates of a protein synthesis-inhibiting drug. In: Borrebaeck CAK, Larrick JW, eds. *Therapeutic Monoclonal Antibodies.* New York: Stockton Press; 1990:143-158.
9. Expression of normal myeloid-associated antigens by acute leukemia cells. *Blood* 1986; 67:1048-1053.

Volume 9

10. Expression of myeloid differentiation antigens in acute non-lymphocytic leukemia: increased concentration of CD33 antigen predicts poor outcome. *Med. Ped. Onc.* 1992; 20:192-200.
11. Expression of lymphoid-associated cell surface antigens by childhood acute myeloid leukemia lacks prognostic significance. *Blood* 1992; 79:2415-2422.
12. Treatment of acute myeloid leukemia cells in vitro with a monoclonal antibody recognizing a myeloid differentiation antigen allows normal progenitor cells to be expressed. *J. Clin. Invest.* 1987; 79:1153-1159.
13. Localization of radiolabeled antimyeloid antibodies in a human acute leukemia xenograft tumor model. *Cancer Res.* 1992; 52:89-94.
14. Letter to FDA from Dr. ID Bernstein to cross-reference IND "1-131 or 1-125 monoclonal antibody to the myeloid p67 antigen".
15. Human hematopoietic precursors in long-term culture: single CD34+ cells that lack detectable T cell, B cell, and myeloid cell antigens produced multiple colony-forming cells when cultured with marrow stromal cells. *J. Exp. Medicine.* 1990; 172:355-358.
16. Precursors of colony-forming cells in humans can be distinguished from colony-forming cells by expression of CD33 and CD34 antigens and light scatter properties. *J. Exp. Med.* 1989; 169:1721-1731.
17. Preparation of soluble CD33. *Celltech Research Study Report.* September 1994: 1-10.
19. Ontogeny of hematopoietic stem cell development: reciprocal expression of CD33 and a novel molecule by maturing myeloid and erythroid progenitors. *Blood.* 1993; 82:792-799.
20. Differences in the frequency of normal and clonal precursors of colony-forming cells in chronic myelogenous leukemia and acute myelogenous leukemia. *Blood.* 1992; 79:1811-1816.
21. M6.1 report on the CD33 cluster workshop: biochemical and genetic characterization of gp67. In: *Leucocyte Typing IV*, ed. W. Knapp et al, Oxford: Oxford University Press; 1989, 814-81.
25. In vitro measurement of avidity of radioiodinated antibodies. *Nucl. Med. Biol.* 1987; 14:605-610.
28. Calicheamicins, a novel family of antitumor antibiotics, 1: Chemistry and partial structure of calicheamicin γ 1¹. *J. Amer. Chem. Soc.* 1987; 109: 3464-3466.

29. Calicheamicins, a novel family of antitumor antibiotics, 2: Chemistry and structure of calicheamicin $\gamma 1^I$. J. Amer. Chem. Soc. 1987; 109:3466-3468.
30. Calicheamicin $\gamma 1^I$ and DNA: Molecular recognition process responsible for site-specificity. Science. 1989; 244:697-699.
31. Characterization of the in vitro cyclization chemistry of calicheamicin and its relation to DNA cleavage. J. Am. Chem. Soc. 1990; 112:4554-4556.
32. Specific abstraction of the 5'(S)- and 4'-deoxyribosyl hydrogen atoms from DNA by calicheamicin $\gamma 1^I$. J. Am. Chem. Soc. 1992; 114:9200-9202.
33. Calicheamicin $\Theta 1^I$: A rationally designed molecule with extremely potent and selective DNA cleaving properties and apoptosis inducing activity. Angew. Chem., Int. Ed. Engl. 1994; 106:183-186.
34. Modulation of nicotinamide adenine dinucleotide and poly(adenosinediphosphoribose) metabolism by calicheamicin $\gamma 1^I$ in human HL-60 cells. Cancer Lett. 1990; 50:141-147.
35. The disulfide calicheamicins. (calicheamicin isolation)
36. Nucleophilic displacements by thioanions on trisulfides. Proc. Chem. Soc. 1962:18-19.
37. Determination of the in vivo redox status of cysteine, cysteinylglycine, homocysteine, and glutathione in human plasma. Anal. Biochem. 1992; 200:218-229.

Volume 10

38. Historical aspects of glutathione and cancer chemotherapy. Pharm. Ther. 1991; 49: 125-132.
39. A study of the reaction of calicheamicin $\gamma 1^I$ with glutathione in the presence of double-stranded DNA. J. Am. Chem. Soc. 1994; 116:1255-1271.
40. The glutathione disulfide of calicheamicin gamma and related disulfides.
41. Antibody conjugates for the delivery of compounds to target sites. US Patent No. 4,671,958; 1987.
42. Calicheamicins, a novel family of antitumor antibiotics. 4. Structure elucidation of calicheamicins $\beta 1^{Br}$, $\gamma 1^{Br}$, $\alpha 2^I$, $\alpha 3^I$, $\beta 1^I$, $\gamma 1^I$, and $\delta 1^I$. J. Am. Chem. Soc. 1992; 114:985-997.
43. The preparation and characterization of monoclonal antibody conjugates of the calicheamicins: a novel family of antitumor antibiotics.
45. New coupling agents for the synthesis of immunotoxins containing a hindered disulfide bond with improved stability in vivo. Cancer Res. 1987; 47:5924-5931.
46. Variation of disulfide stability for CTM01-calicheamicin hydrazide conjugates.
47. Enhanced stability in vitro and in vivo of immunoconjugates prepared with 5-methyl-2-iminothiolanone. Bioconjugate Chem. 1994; 5:248-256.
49. A brief survey of methods for preparing protein conjugates with dyes, haptens, and cross-linking reagents. Bioconjugate Chem. 1992; 3:2-13.
50. Preparation of genetically engineered monoclonal antibodies for human immunotherapy. Hum. Antibod. Hybridomas. 1992; 3:137-145.
51. Protein Engineering of Antibodies. Crit. Rev. Biotech. 1992; 12:437-462.

Volume 11

52. New approaches to non-immunogenic monoclonal antibody cancer therapies. Annual Reports in Med. Chem. 1993; 28:237-246.
53. The preparation and characterization of hybrid conjugates prepared from the calicheamicins and monoclonal antibodies.
58. CD33 Section Report. IN: Leucocyte typing V. Eds, Schlossman SF, et al. Oxford: Oxford University Press: (in press).

Volume 21-22

70. A six week intravenous toxicity study of CL 184,538 (N-acetyl-gamma-dimethyl-hydrazide derivative of calicheamicin), an anticancer agent, in rats (Study 92080).

Volume 22-23

71. A six week intravenous toxicity study of, CL 184,538 (N-acetyl-gamma-dimethyl-hydrazide derivative of calicheamicin), an anticancer agent, in dogs (92081).

Volume 26

74. A single dose intravenous toxicity study of CL 190,396 (N-acetyl-epsilon derivative of calicheamicin), an anticancer agent, in dogs (Study 92049).

(note for studies 75-85, 87 that γ_1^1 -calicheamicin (CL 181,441) is impossible to generate from the drug product and is not present during synthesis)

Volume 27

75. An acute intravenous toxicity study of E33288 gamma-1-I in mice (Study 86153).
76. An acute intravenous toxicity study of E33288-gamma-1-I (CL 181,441) in mice with a five week Observation period (Study 86204).

Volume 27-28

77. An acute intravenous toxicity study of E33288-gamma-1-I in rats (Study 86283).

Volume 28

78. A comparison study of the hepatic effects of E33288- gamma-1-I (CL 181,441) in rats (Study 88025).
79. Magnetic resonance imaging (MRI) study on rats dosed with E33288 (CL 181,441, calicheamicin) (Study 88077).

Volume 29

80. A single-dose topical and subcutaneous study of E33288- gamma-1-I in rats, with a 120 day observation period (Study 87141).
81. An acute dermal toxicity study of E33288 gamma-1-I (CL 181,441) in rats (Study 86205).
82. A single-dose intranasal study of E33288 gamma-1-I in rats, with a 120 day observation period. final report (Study 87142).

Volume 30-31

83. An acute intravenous toxicity study of E33288-gamma-1-I (CL 181,441) in cynomolgus monkeys (Study 88024).
84. A 5 day intravenous toxicity study of E33288 gamma-1-I in rats with an observation period of several months (Study 87136).

Volume 32

85. A 5 Cycle (21 day interval) intravenous toxicity study of E33288-gamma-1-I in rats (Study 87137).

Volume 33

87. Effects of gamma calicheamicin, CL 181,441 on murine bone marrow hematopoietic colony formation in vitro (Study 92119).
88. Exploratory studies on the in vitro toxicity of gamma calicheamicin (CL 181,441), NAc-gamma calicheamicin (CL 181,927), NAc-Gamma calicheamicin DMA (CL 186,760), Calicheamicin NAc-gamma DMH (CL 184,538), and NAc-epsilon calicheamicin (CL 190,396) (Study 10192).

Studies Previously Reviewed-----none

Note that portions of this review were excerpted directly from the sponsor's submission.

I. PHARMACOLOGY

22. Anti-CD33-N-Ac gamma calicheamicin inhibits colony forming activity from blood and bone marrow of diagnostic AML patient samples. Conducted by [] Frozen mononuclear cells separated by density centrifugation of marrow from normal subjects and pediatric and adult AML patients were tested for colony forming ability in semisolid media (methylcellulose). Cells were incubated with hP67.6 conjugate for 2 hr and washed prior to plating. Fifty-three percent (8/15) of the pediatric AML samples had $\geq 60\%$ inhibition of colony formation at 2 ng/ml of hP67.6 conjugate. Forty-four percent (12/27) of the adult AML samples had

≥60% inhibition of colony formation at 10 ng/ml of hP67.6 conjugate. FAB classification, CD33, CD34, and MDR-1 expression did not predict which samples were sensitive to inhibition.

23. Binding characteristics of hP67.6 antibody and hP67.6 conjugate to normal human peripheral blood leukocytes and bone marrow cells: comparative flow cytometric analysis. Conducted by [] No significant differences were found in the binding specificity (cellular distribution) of murine P67.6, humanized P67.6, or hP67.6 conjugate. Conjugate formation caused a minor decrease in binding affinity.

26. Evaluation of relative immunoaffinities of four batches of hP67.6-N-acetyl γ_1^I calicheamicin dimethyl AcBut (CL 555,201). Conducted by [] The binding affinities of four batches of hP67.6 conjugate were compared to mP67.6 and hP67.6 by competitive radio-immunoassay. The humanized form had about 1/2 the binding affinity of the mP67.6. No differences were noted between hP67.6 and the four batches of conjugate.

27. Evaluation of hydrazide-, amide-, and "hybrid"-linked conjugates of N-acetyl γ_1^I calicheamicin (CL181,927) with murine and humanized versions of the anti-CD33 antibody, P67.6, against the HL-60 tumor as a model of acute myeloid leukemia (AML). Conducted by [] The conjugate of the murine P67.6 with a hydrazone linkage was 7000-fold more cytotoxic to HL-60 cells in culture than the conjugate with an amide linkage. Likewise the hydrazone conjugate was superior *in vivo* against HL-60 xenografts; 450 and 900 μ g/kg doses given every 4 days starting 7 days after implantation produced 9/10 tumor-free survivors. This suggests that reduction of the disulfide to release drug is a minor pathway in cells compared to hydrolysis. Development of a modified linker that retained the hydrazone functional group yet attached to lysines on the antibody (so that the adverse oxidative conditions required for attaching hydrazone to glycosyl groups could be avoided) led to the AcBut linker. The IC_{50} of the hP67.6 conjugate against HL-60 cells was <0.1 ng/ml. Three doses of hP67.6 conjugate produced 5/5 tumor free survivors at 150, 450, and 900 μ g/kg in mice bearing HL-60 xenografts. A single dose produced 2/5 tumor free survivors at 250 μ g/kg and 3/5 tumor-free survivors at 500 and 750 μ g/kg. hP67.6 conjugate also caused complete regression of established tumors (200 mg) after 3 doses of 150, 450, or 900 μ g/kg.

55. In vitro cellular cytotoxicity of NAc-calicheamicin gamma (CL181,927), NAc-calicheamicin gamma DMH (CL184,538), NAc-calicheamicin gamma DMH AcBut Acid (CL191,305), and hP67.6-NAc-calicheamicin gamma DMH AcBut conjugate (CL 555,201). Conducted by [] Ability to inhibit [3 H]thymidine incorporation was assessed in CD33⁺ HL-60 cells or CD33⁻ Raji cells after 3 days of growth. Drug was added for the 1st hr or for 3 days of continuous exposure. The conjugate showed high specificity to CD33⁺ cells and was much more potent than free calicheamicin.

	1 hr IC_{50} (ng/ml)		3 day IC_{50} (ng/ml)	
	HL-60	Raji	HL-60	Raji
NAc-calicheamicin DMH	9.2	4.7	0.092	0.17
NAc-calicheamicin AcBut	18.6	16.4	0.87	1.1
NAc-calicheamicin	0.96	0.25	0.091	0.082
hP67.6 conjugate	0.00046	35.7	<0.0005	0.61

44. Synthesis and in vitro cellular cytotoxicity of epsilon calicheamicin (CL 181,287) and N-acetyl epsilon calicheamicin (CL 190,396). Conducted by [] e-Calicheamicin is the structure remaining after γ_1^I -calicheamicin has been triggered and abstracted hydrogen atoms to quench the diradical. N-Ac-e-calicheamicin was 4000-fold less potent than N-Ac- γ -calicheamicin against Raji cells as determined by [3 H]thymidine incorporation. e-Calicheamicin was 10800-fold and 5300-

fold less potent than γ -calicheamicin against A2780 and A2780/DDP (cisplatin-resistant) human ovarian cells. Curiously, the A2780/DDP cells were 4-fold and 8-fold cross-resistant to the ϵ - and γ -calicheamicins respectively.

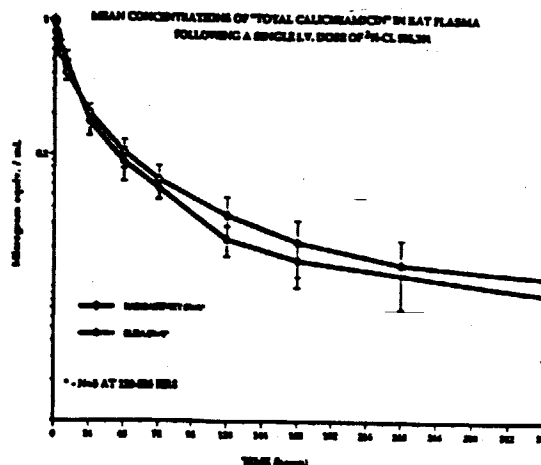
II. PHARMACOKINETICS AND TOXICOKINETICS

Toxicokinetic data was also provided for studies #63, 64, 65, 68, 69, and 72 and is reviewed under those studies in the Toxicology section. That data is included in the Pharmacokinetics Summary below.

89. Pharmacokinetics of the hP67.6³H-N-Acetyl- γ -DMH-AcBut calicheamicin conjugate (CL 555,201) following a single intravenous administration in rats. (Study A9442). Conducted by [] Four rats received 1.6 mg/kg hP67.6 conjugate containing [³H]N-Acetyl- γ ₁¹-calicheamicin DMH AcBut. Plasma was analyzed for radioactivity and for "total calicheamicin" by ELISA. Less than 2% of the drug was free at 120 hr after dosing. The pharmacokinetic profile and derived parameters were comparable by both methods of calculation, providing further validation of the ELISA assay.

Comparative Pharmacokinetic Parameters

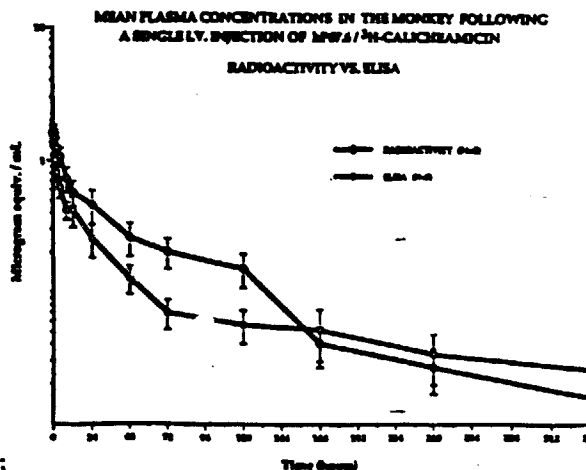
	[³ H]	ELISA
C _{max} (μg/ml)	1.0	1.28
AUC _{0-∞} (μg·hr/ml)	21.2	18.9
Cl _t (ml/min/kg)	0.037	0.042
t _{1/2} (hr)	95	66



90. Pharmacokinetics of the hP67.6³H-N-Acetyl- γ -DMH-AcBut calicheamicin conjugate (CL 555,201) following a single intravenous administration in cynomolgus monkeys (Study A9452). Conducted by [] Five cynomolgus monkeys received 1.3 mg/kg hP67.6 conjugate containing [³H]N-Acetyl- γ ₁¹-calicheamicin DMH AcBut. Plasma was analyzed for radioactivity and for "total calicheamicin" by ELISA. Less than 2% of the drug was free at 120 hr after dosing. The pharmacokinetic parameters were not always comparable by both methods of calculation. The AUC by ELISA was ~70% of the radioactivity value and the t_{1/2} was 36% greater.

Comparative Pharmacokinetic Parameters

	[³ H]	ELISA
C _{max} (μg/ml)	1.62	1.54
AUC _{0-∞} (μg·hr/ml)	46.4	29.3
Cl _t (ml/min/kg)	0.016	0.023
t _{1/2} (hr)	119	162



91. Validation of an ELISA assay for quantitation of total calicheamicin derivatives in rat plasma and cross-validation for such quantitation in monkey plasma. Study TSD358 and TSD260.2) Conducted by

Rat or monkey plasma (100 μ l) was treated with 100 μ l of 10% mercaptoethanol for 1 hr at 37°C to release calicheamicin from the conjugate. The reaction was quenched with 50 μ l 0.37 g/ml iodoacetamide, and the released drug was extracted with 1 ml acetone. The standard was calicheamicin DMH. The principle of the ELISA assay is the ability of the extract to inhibit the binding of peroxidase conjugated drug to rabbit anti-calicheamicin antibody adsorbed to a microtiter plate. Unknown values were calculated with a 6-parameter logistic model.

The LOQ was 8.7 ng/ml at which level the CV was 5.5%. The calibration range was 8.7 to 2600 ng/ml and the response was linear ($r=0.998$). The CV within a set of replicates was always <20% and between 6 separate rat plasmas was 20.9%. Accuracy varied from 0-50%. Monkey plasma gave ~50% higher values when compared against standards in rat plasma. Frozen samples were stable for 5 mo and could withstand one freeze/thaw cycle. Standing at room temp for 4 hr significantly increased the observed concentration. Rhamnose, the calicheamicin sugar residue, did not interfere. The variation between days was $\leq 10\%$. The assay was not subject to edge effects or inter-plate effects.

92. Validation of an ELISA assay for quantitation of total antibody (hP67.6) in rat plasma and cross-validation for such quantitation in monkey plasma. Study TSD358 and TSD 260.2) Conducted by

Rat or monkey plasma (100 μ l) is diluted 1:10, 1:20, 1:40, and 1:80 in assay buffer. The standard was hP67.6. The principle of the ELISA assay is the ability of the sample to bind to CD33 antigen adsorbed to a plate. The bound antibody is detected with murine anti-IgG₄ conjugated to peroxidase. Unknowns were calculated with a 6-parameter logistic model.

The LOQ was 100 ng/ml at which level the CV was 4.4% but the error was -49%. The calibration range was 100 to 100,000 ng/ml and the response was linear ($r=0.997$). The CV within a set of replicates was always <32% and between 6 separate rat plasmas was 7.4%. The accuracy, however, was -20-30% for 1000 to 100,000 ng/ml. Monkey plasma gave ~20% lower values when compared against standards in rat plasma. Samples were unstable at 4°C. Frozen samples were stable for 5 mo at -70°C and could withstand one freeze/thaw cycle. Anti-CD33 IgG₁ and non-specific IgG₄ gave background readings. The variation between days was 26-32%. Standing at room temp for 4 hr significantly decreased the observed concentration. The assay was not subject to edge effects or inter-plate effects, but an effect of timing was seen (wells that received all the reagents first developed greater absorbance than wells that were the last to receive all their reagents).

SUMMARY OF PHARMACOKINETICS

The pharmacokinetic parameters for the drug substance, hP67.6 conjugate, are shown in the following table. Very little dissociation of calicheamicin occurred *in vivo*. The $t_{1/2}$ was very long (3-7 days) as appropriate for an immunoglobulin. The $t_{1/2}$ in monkeys was much greater than in rodents.

Pharmacokinetic Parameters for hP67.6 Conjugate

Study #	species	dose μ g/kg	C _{max} μ g/ml		AUC μ g•hr/ml		t _{1/2} hr		% Free (time) calicheamicin
			Total drug	MoAb	Total drug	MoAb	Total drug	MoAb	
89	rat	1600	1.14	-	19.6	-	81	-	<2 (120 hr)
90	monkey	1300	1.58	-	37.9	-	141	-	<2 (120 hr)
63	rat	1200	0.494	31.7	8.78	1180	39	72	3.6
64	monkey	1800	1.49	3.57	25.7	2530	183	176	0.8
65	chimp	42 ^a	7.97	-	-	-	-	-	-

^a double the starting dose in humans on a mg/m² basis

Calicheamicin analogues had half-lives of ~18 hr in rats and dogs. Addition of the AcBut linker to the DMH derivative elevated the C_{max} 20-fold and the AUC ~10-fold. A dose of 1000 $\mu\text{g/kg}$ of 184,538, which was non-lethal to rats, produced a plasma concentration that was 10-20-fold higher than the 1 hr IC_{50} against HL-60 cells. Possible gender-based differences in the pharmacokinetics were noted.

Pharmacokinetic Parameters for Calicheamicin Compounds

Study #	compound #	derivative	species	dose $\mu\text{g/kg}$	C_{max} ng/ml	AUC $\mu\text{g}\cdot\text{hr/ml}$	$t_{1/2}$ hr
68	184,538	DMH	rat σ	1000	161	0.628	14.5
			φ		116	0.355	27
69	184,538	DMH	dog σ	250	25	0.107	21.8
			φ		40	0.231	17.1
72	191,305	DMH AcBut	rat σ	1000	2,350	3.76	16.2
			φ		2,880	4.42	13.1
73	190,396	NAc- ϵ	rat σ	1000	90	-	-
			φ		57	-	-

III. TOXICOLOGY

A. Single Dose Toxicity

60. A single dose intravenous toxicity study of CL 555,201 (N-acetyl-gamma- dimethyl-hydrazide-acetyl-butyryloxy derivative of calicheamicin [CL 191,305] conjugated to hP67.6 [CL 555,001]), an anticancer agent, in rats (Study 93119). Conducted by _____ according to GLP.

species: Sprague Dawley rats (5/sex/group)
 drug: hP67.6 conjugate
 dosage- hP67.6 equivalents: 0, 0.4, 4, and 12 mg/kg -
 toxic equivalents: 0, 10, 100, and 300 $\mu\text{g/kg}$ NAc-calicheamicin AcBut (191,305)
 age; weight: 8 weeks; 252-302 g σ , 177-216 g φ
 observation: 16 days
 route: i.v. via tail vein

Observations

Clinical signs twice/daily
 Body weights before dosing, day 3, 6, 13
 Food consumption before dosing, day 3, 6, 13
 Urinalysis day 3
 Gross pathology termination

- a. Clinical signs: mortality: 6/10 at MD all sacrificed moribund days 3 (5) and 6 (1); 10/10 at HD on days 2-3, 2 sacrificed moribund
 LD: 1 feces

- MD, HD: prostration, hunched posture, ↓ activity, pale and jaundiced, orange urine, stained perianal area, ↓ feces, chromodacryorrhea, chromorhinorrhea, dyspnea, tachypnea, lacrimation, ptosis, hypothermia
- b. Body weight: MD- weight loss days -1 to 6, surviving animals gained weight faster than controls during week 2
- c. Food consumption: ↓ days -1 to 6 at MD; no effect at LD
- d. Urinalysis: HD 4+ protein, 3+ bilirubin, 3+ blood, 2+ urobilinogen
MD 2+ to 4 protein, 2+ to 3 bilirubin, 3 blood, 2+ to 4 urobilinogen
- e. Gross Pathology:
MD, HD animals that expired: rough liver surfaces; dose-related incidence of yellow salivary gland, skeletal muscle, kidneys, thymus, prostate, seminal vesicles, uterus, lymph nodes, urinary bladder, adipose tissue, and skin; red foci in stomach; red areas in heart, lungs, thymus, and testes
MD animals that survived: mottled liver in 1/3 ♀, rough spleen in 1/3 ♀, white spleen in 2/4 total

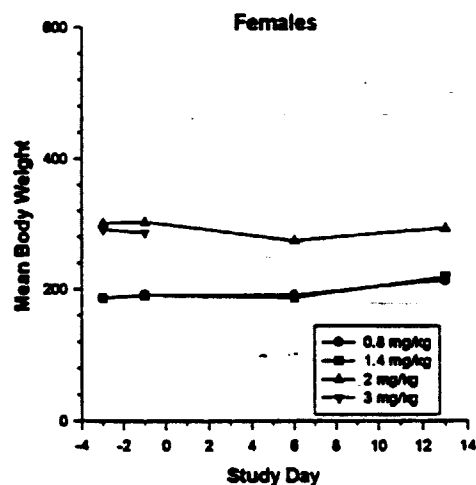
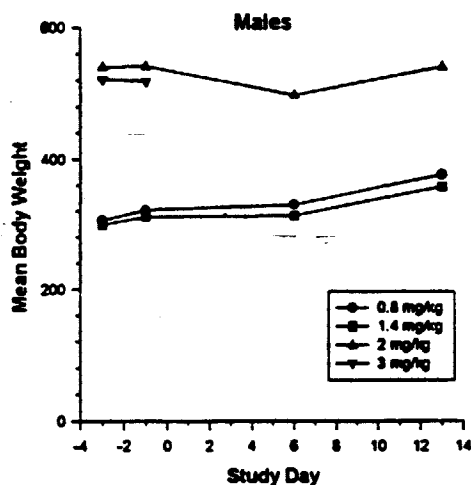
61. An exploratory single dose intravenous tolerance study of CL 555,201 (N-acetyl-gamma-dimethyl-hydrazide-acetyl-butyryloxy derivative of calicheamicin [CL 191,305] conjugated to hP67.6 [CL 555,001]), an anticancer agent, in rats (Study 94033). An unaudited study conducted by []

species: Sprague Dawley rats (5/sex/group)
drug: hP67.6 conjugate
dosage- hP67.6 equivalents: 0.8, 1.4, 2.0 and 3.0 mg/kg
toxic equivalents: 20, 35, 50, and 75 µg/kg NAc-calicheamicin AcBut (191,305)
age; weight: 0.8, 1.4 mg/kg: 9 weeks; 291-352 g ♂, 179-202 g ♀
2, 3 mg/kg: 17 weeks; 481-630 g ♂, 256-350 g ♀
observation: 15 days
route: i.v. via tail vein

Observations

Clinical signs	daily
Body weights	before dosing, day 6, 13, 15
Food consumption	before dosing, day 6, 13, 15
Hematology -	days 3, 14
Clinical chemistry	days 3, 14
Urinalysis	day 3, 14
Gross pathology	at sacrifice (organs p. 15)
Histopathology	at sacrifice on liver, kidneys, gross lesions, and some bone marrows only

- a. Clinical signs: mortality: 5/10 at 2 mg/kg on days 3-6, 10/10 at 3 mg/kg on day 3
0.8 mg/kg: ↓ feces, stained perianal area
1.4 mg/kg: ↓ feces, ↓ activity
2 mg/kg: ↓ feces, ↓ activity, stained perianal area, unkempt appearance, orange urine, jaundice, pallor
3 mg/kg: ↓ activity, jaundice, pallor, orange urine, stained perianal area, ↓ feces, ptosis, unkempt appearance
- b. Body weight: see figures



- c. Food consumption: slight ↓ during 1st week in 0.8, 1.4, and 2 mg/kg groups but considered not toxicologically significant
- d. Hematology: (note changes are relative to 0.8 mg/kg group)
 90-95% ↓ platelet counts in ♂ and ♀ at ≥ 2 mg/kg and 44% ↓ in ♀ at 1.4 mg/kg; 5-15% ↓ mean HCT at 3 mg/kg; 12-20% ↓ HGB at 3 mg/kg; 42% ↓, 72% ↓ reticulocytes in ♂, ♀ at 3 mg/kg; ♀ also had 48% and 65% ↓ reticulocytes at 1.4 and 3 mg/kg
- e. Clinical chemistry: AST ↑ 93 and 210-fold at 2 and 3 mg/kg which resolved in surviving animals by day 14; ALT ↑ 129 and 365 fold at 2 and 3 mg/kg which was ↓ 2-fold at day 14 in surviving animals; total bilirubin ↑ 36 and 79-fold at 2 and 3 mg/kg which resolved by day 14; ♀ at 1.4 mg/kg also had 9-fold ↑ AST and 21-fold ↑ ALT
- f. Urinalysis:
 day 3
 2 mg/kg 4+ proteinuria, 4+ hematuria, 1 to 2+ urobilinogen
 3 mg/kg 4+ proteinuria, 4+ hematuria, 2+ bilirubin,
 day 14 2 mg/kg 2 to 4+ proteinuria
- g. Gross Pathology:
 early deaths: mottled red and/or rough liver surfaces, tan kidneys, yellow discoloration of multiple organs (jaundice); discolored foci in stomach mucosa
 surviving animals: tan kidneys in 2 mg/kg animals
- h. Histopathology: on liver, kidneys, gross lesions, and some bone marrows only
 early deaths: hepatocellular necrosis, hepatocellular karyocytomegaly, periportal and sinusoidal mixed cell infiltrate, liver congestion; oval cell/bile duct proliferation in 1 ♀ at 2 mg/kg; autolytic changes in kidney obscured changes related to drug substance
 surviving animals:
 0.8 mg/kg: kidney tubular dilation and vacuolation, eosinophilic granules, large amorphous hyaline droplets
 1.4 mg/kg: single cell necrosis, hepatocellular karyocytomegaly, hepatocyte vacuolation, hemosiderin in Kupffer cells, oval cell/bile duct proliferation; kidney tubular dilation, basophilia
 2 mg/kg: single cell necrosis, hepatocellular karyocytomegaly, hepatocyte vacuolation, hemosiderin in Kupffer cells, oval cell/bile duct proliferation; kidney tubular dilation, casts, karyocytomegaly, basophilia

62. A single dose intravenous toxicity study of CL 555,201 (N-acetyl-gamma-dimethyl-hydrazide-acetyl-butyryloxy derivative of calicheamicin [CL 191,305] conjugated to hP67.6 [CL 555,001]), an anticancer agent, in male monkeys (Study 93214). Conducted by [redacted] according to GLP. A 3 mg/kg dose of hP67.6 conjugate was tolerated with no overt toxicities, but 4.5 mg/kg or higher killed half the animals and produced marked clinical signs, reddening of multiple tissues, and alterations in liver appearance.

species:	♂ monkeys (<i>Macaca fascicularis</i>) (4/group)
drug:	hP67.6 conjugate
dosage- hP67.6 equivalents:	0, 3, 4.5, and 6 mg/kg
toxic equivalents:	0, 75, 112.5, 150 µg/kg NAc-calicheamicin AcBut (191,305)
age; weight:	2-5 yr, 3-5.1 kg
observation:	16-23 days
route:	i.v. via saphenous vein

Observations

Clinical signs	daily (mortality- twice/daily)
Body weights	day -1, 6, 13, 20
Appetite	daily
Urine color	day of dosing
Gross pathology	dead or pre-terminal sacrificed only (organs p. 14)

- a. Clinical signs:
- 4.5 mg/kg: two sacrificed moribund days 7,8; emesis, soft feces, ↓ activity, hunched appearance, pale gums, rales, dyspnea, chromorhinorrhea, injected sclera, tail chewing, labored breathing, red crust on nares and hands and clot in oral cavity, brown discolored incisors
- 6 mg/kg: 2/4 died on day 17; emesis, soft feces, ↓ activity, hunched appearance, atonia, pale gums, hypothermia, unresponsive eyes multifocal brown circular lesions on thighs, and ankle; shoulder ulceration; pale and dry mucous membranes, empty GI tract
- b. Body weight: weight loss in 2/4 at 6 mg/kg
- c. Appetite: at 6 mg/kg appetite declined to poor by day 4; at 4.5 mg/kg appetite started to decline to poor by day 6
- d. Gross pathology: rough surface, mottling, or reticular pattern in livers; red gall bladders
- 4.5 mg/kg: red-tinged fluid (hemorrhage) in abdominal cavity, reddening of retroperitoneal area, red areas in lungs, foamy red contents in lungs, red areas in stomach and intestinal tract, red petechiae in stomach, red mesentery, red kidney capsule
- 6 mg/kg: red-tinged fluid (hemorrhage) in abdominal cavity, red foci in heart, reddened pericardium, red diaphragms, red areas in lungs, red areas in stomach and intestinal tract, red mesentery, red kidney capsule, red areas in bladder, red seminal vesicles

65. An exploratory single dose intravenous tolerance study of CL 555,201 (N-acetyl-gamma-dimethyl-hydrazide-acetyl-butyryloxy derivative of calicheamicin [CL 191,305] conjugated to hP67.6 [CL 555,001]), an anticancer agent, in the chimpanzee (Study 94118). A non-GLP study conducted by [redacted] Increases in WBC, AST, and ALT were noted, but it is unknown whether these were related to the ketamine/isoflurane anesthesia. No other toxic signs were noted.

species:	♂ chimpanzees (2); non-naive but not previously exposed to humanized or murine monoclonal antibodies
drug:	hP67.6 conjugate
dosage hP67.6 equivalents:	0.5 mg/m ²

toxic equivalents: 12.5 $\mu\text{g}/\text{m}^2$ NAc-calicheamicin AcBut (191,305)
 age; weight: 18-30 yr; 59-66 kg
 observation: 2 wk
 route: 2 hr i.v. infusion via cephalic vein

Observations

Clinical signs daily (mortality- twice/daily)
 Appetite daily
 Body Temperatures days -5, -1, 0, 1, 3, 7, 10, and 14
 Hematology days -5, -1, 3, 7, 10, and 14
 Serum Chemistry days -5, -1, 3, 7, 10, and 14
 Pharmacokinetics 0, 2, 4, 26, 74, 170, 242, and 338 hr from start of infusion (conducted by
) by ELISA for total antibody, unconjugated
 calicheamicins, and total calicheamicins

- a. Clinical signs, Appetite, Body temp: no treatment related effects
 b. Hematology: 75 and 82% \uparrow WBC on day 1-3
 c. Clinical Chemistry: 129 and 101% \uparrow ALT on days 1-3; 350 and 208% \uparrow AST on days 1-3; also trend for 15% \uparrow globulin
 d. Pharmacokinetics: Unconjugated calicheamicin derivatives were not detectable (limit of quantitation = 8.7 ng/ml). Total calicheamicin (conjugated + unconjugated) were 6.27, 3.2 ng/ml immediately after infusion start. Total antibody could not be assayed due to interference from chimpanzee plasma.

Total Calicheamicin Derivatives (ng/ml) at Specified Times After Infusion Start

animal	pre	2 hr	4	26	74	168	240	336
X124	1.69 ^b	(4.89) ^a	(3.87) ^a	-	-	-	-	-
019	1.70 ^b	7.97	6.71	-	-	-	(3.55) ^a	-

^a not considered different from background

^b background in chimp plasma

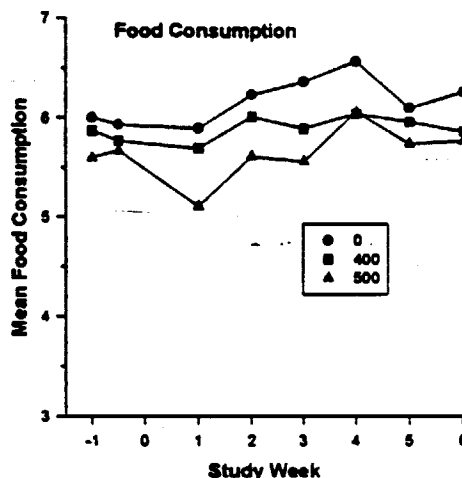
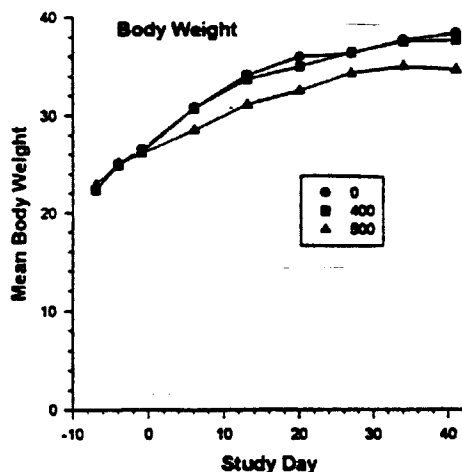
67. A single intravenous dose exploratory study of calicheamicin (CAL) analogs (antitumor antibiotics) in mice (Study 90126). A non-GLP study conducted by [] Mice were injected with 184,538 or 186,029. Only the data on 184,538 (NAc-calicheamicin- γ -l-I-DMH) was reviewed as trace amounts are potentially present in the drug product and it would be similar in structure to calicheamicin equivalents that might be released from the hP67.6 conjugate.

species: CD-1 σ mice (10/group)
 drug: 184,538
 dosage: 0, 50, 100, 300, 400, and 500 $\mu\text{g}/\text{kg}$
 age; weight: 5 weeks; 23-32 g
 observation: days
 route: i.v. via tail vein

Observations

Clinical signs daily
 Body weights before dosing, weekly
 Food consumption before dosing, weekly
 Gross pathology termination
 Histopathology liver, kidney only

- a. Clinical Signs: no mortality; ↓ fecal output at 500 µg/kg
 b. Body Weight, Food consumption: 10% ↓ body weight in 500 µg/kg group days 6-41; ~10% ↓ food consumption in 500 µg/kg group days week 1-4 and 8% in 400 µg/kg group at week 4



- c. Gross Pathology: ↓ mean absolute and relative liver weights at ≥300 µg/kg, rough liver surface in 2/10 at 400 µg/kg and 5/10 mice at 500 µg/kg
 d. Histopathology: hepatocellular pleomorphism and nodular regeneration at ≥300 µg/kg

68. A single dose intravenous toxicity study of CL 184,538 (N-acetyl-gamma-dimethyl- hydrazide derivative of calicheamicin), an anticancer agent, in rats (Study 92044). Conducted by [] according to GLP.

species: CD rats (5/sex/group)
 drug: 184,538
 dosage: 0, 10, 100, 300, and 1000 µg/kg NAc-calicheamicin DMH (184,538)
 age; weight: 7 weeks; 211-268 g ♂, 155-190 g ♀
 observation: 47 days
 route: i.v. via tail vein

Observations

Clinical signs	twice/daily
Body weights	before dosing, twice/wk
Food consumption	before dosing, twice/wk
Urinalysis	days 1-14
Hematology	days 12 and 40
Clinical Chemistry	days 12 and 40
Gross pathology	termination (organs p. 16)
Histopathology	termination
Toxicokinetics	0.5, 2, 6, 8, 12, and 24 hr post dosing

- a. Clinical signs: mortality 10/10 at 300 (days 4-6); 10/10 at 1000 (day 4); prior to-death animals were hypoactive, prostrate, unkempt, diarrhea, no feces, chromodacryorrhea, wet perianal area
 100: soft, liquid, few or no feces
 b. Body weights: ↓10% and 30% prior to death in 300 and 1000 µg/kg groups respectively; ↓10% for wk 1-2 in ♂ and wk 1 in ♀ in 100 µg/kg groups

- c. Food consumption: at 100 µg/kg 125-40% during wk 1
- d. Urinalysis: hemoglobinuria due to 4% ethanol vehicle
- e. Hematology: in 100 µg/kg ♀: 15% Hct, 17% RBC, 152% lymphocyte counts (day 12)
in 100 µg/kg ♂: 133% WBC and 143% lymphocyte count on day 12
- f. Clinical Chemistry: no treatment related changes
- g. Gross pathology: no treatment related changes in 10 and 100 µg/kg groups;
in 300 and 1000 µg/kg animals that died a small spleen, thymus, and seminal vesicle; enlarged adrenal gland; and ulcerated and distended stomach were noted
- h. Histopathology: hepatocellular karyocytomegaly at ≥ 100 µg/kg; single cell liver necrosis at 1000 µg/kg; karyocytomegaly in kidney cortical tubular epithelium at ≥ 100; lymphoid depletion in spleen and thymus at ≥ 300; extramedullary hematopoiesis in spleen at ≥ 300; bone marrow hypocellularity at ≥ 300; ulcerated stomach, inflamed nonglandular stomach, and acanthosis at ≥ 300
- i. Toxicokinetics: Levels were below the LOQ of 2.5 ng/ml for the 10 and 100 µg/kg groups, and for 300 µg/kg ♂ and ♀ after 12 and 2 hr respectively.

Toxicokinetic Parameters for 184,538

dose	300 µg/kg		1000 µg/kg	
	♂	♀	♂	♀
C _{max} (µg/ml)	0.037	0.010	0.161	0.116
AUC _{0-t} (µg·hr/ml)	-	-	0.628	0.355
t _{1/2} (hr)	-	-	14.5	27.0

69. A single dose intravenous toxicity study of CL 184,538 (N-acetyl-gamma-dimethyl- hydrazide derivative of calicheamicin), an anticancer agent, in dogs (Study 92047). Conducted by

species: beagle dogs (2/sex/group)
 drug:- 184,538
 dosage: 0, 2.5, 25, 75, and 250 µg/kg NAc-calicheamicin DMH (184,538)
 age, weight: 6 mo; 7.9-10.1 kg ♂, 7.3-8.4 kg ♀
 observation: 45 days
 route: slow i.v. bolus via cephalic vein

Observations

Clinical signs twice/daily
 Body weights before dosing, twice/wk
 Food consumption before dosing, daily
 Urinalysis days 1-14
 Hematology pre-dose, days 6, 13, 22, 38
 Clinical Chemistry pre-dose, days 6, 13, 22, 38
 Gross pathology termination (organs p. 28)
 Histopathology termination
 Toxicokinetics 0.5, 1, 2, 6, 8, and 12 hr post dosing

- a. Clinical signs: Mortality was 1/4 at 75 (day 3); 4/4 at 250 sacrificed moribund on day 4; prior to death animals had emesis, mucoid feces, red-tinged, bloody feces, hypothermia, excessive salivation, ↓ activity, swollen leg. Surviving animals had soft or loose

feces, hematest positive feces, and emesis at ≥ 25 $\mu\text{g/kg}$. Mucoïd feces and thin appearance at 75 $\mu\text{g/kg}$.

- b. Body weights: 110% in 75 $\mu\text{g/kg}$ groups in 1st week
- c. Food consumption: 159% and 28% in σ^7 and ♀ respectively in 1st week
- d. Urinalysis: no red-tinged urine was noted
- e. Hematology: no treatment related changes
- f. Clinical Chemistry: 110% APTT in ♀ and 120% in surviving σ^7 at 75 $\mu\text{g/kg}$
- g. Gross pathology: No treatment related changes found in surviving animals. Findings in dead animals were multiple reddened areas in mucosa of GI tract, small spleen, and small thymus.
- h. Histopathology: Dead animals had lymphoid depletion in spleen, thymus, and lymph nodes; hypocellular bone marrow; karyocytomegaly in large intestine with congestion, necrosis, and hemorrhage. Surviving animals had regeneration of kidney tubules at 25 and 75 $\mu\text{g/kg}$.
- i. Toxicokinetics: Levels were below the LOQ of 2.5 ng/ml for the 2.5, 25, and 75 $\mu\text{g/kg}$ groups.

Toxicokinetic Parameters for 191,305

dose	250 $\mu\text{g/kg}$	
	σ^7	♀
C_{max} ($\mu\text{g/ml}$)	0.025	0.040
AUC_{0-t} ($\mu\text{g}\cdot\text{hr/ml}$)	0.107	0.231
$t_{1/2}$ (hr)	21.8	17.1

72. A single dose intravenous toxicity study of CL 191,305 (N-acetyl-gamma-dimethyl- hydrazide AcBut derivative of calicheamicin), an anticancer agent, in rats (Study 93026). Conducted by [] according to GLP.

species: CD rats (5/sex/group)
 drug: 191,305
 dosage: 0, 10, 100, 300, and 1000 $\mu\text{g/kg}$ NAc-calicheamicin DMH AcBut
 age, weight: 8 weeks; 282-337 σ^7 , 181-229 ♀ g
 observation: 44 days
 route: i.v. via tail vein

Observations

Clinical signs twice/daily
 Body weights before dosing, once/wk
 Food consumption before dosing, once/wk
 Urinalysis days 1-14
 Hematology days 12 and 40
 Clinical Chemistry days 12 and 40
 Gross pathology termination (organs p. 16)
 Histopathology termination
 Toxicokinetics 0.5, 2, 6, 8, 12, and 24 hr post dosing

- a. Clinical signs: mortality 5/5 σ^7 and 1/5 ♀ at 300 (day 5); 10/10 at 1000 (days 3- 4); prior to death animals were hypoactive, prostrate, 1 activity, 1 fecal production, soft or mucoïd feces, unkempt, chromodacryorrhea
 red-tinged urine in all groups; 300 $\mu\text{g/kg}$ ♀ had wet perianal area, few, soft, or

- liquid feces, ↓ activity, and unkempt appearance during 1st week
- b. Body weights: 19% in ♂ at 10 and 100 µg/kg at day 41 only; ↓ 15% in 300 µg/kg ♀ days 6-41
 - c. Food consumption: ↓ 35% in 300 µg/kg ♀ during 1st week followed by 25% ↓ during 2nd week
 - d. Urinalysis: hemoglobinuria on day 0 in all groups due to vehicle induced hemolysis
 - e. Hematology: no treatment related changes
 - f. Clinical Chemistry: 25% ↑ BUN and 22% ↑ creatinine in 300 µg/kg ♀ on day 40
 - g. Gross pathology: No treatment related changes were found in surviving animals. Early death animals had: a) small spleens and thymuses, pale liver foci, and discolored foci in glandular stomach at ≥ 300 µg/kg; b) dark red femoral marrow at 300 µg/kg; and c) small seminal vesicles at 1000 µg/kg
 - h. Histopathology: Early death animals had kidney tubular dilation, ↑ basophilia, karyocytomegaly of tubular epithelium, and tubular casts; hepatocellular necrosis; atrophy of seminiferous tubules in 1 ♂ at 1000; lymphoid depletion in spleen and thymus; hypocellularity of bone marrow; intraepithelial abscesses in nonglandular epithelium.

Microscopic Findings in Surviving Animals Administered 191,305

µg/kg	100		300
	♂	♀	♀
kidney tubular dilation	1/5	1/5	4/4
↑ basophilia	5/5		4/4
karyocytomegaly of tubular epithelium	4/5		4/4
tubular degeneration	1/5		4/4
liver karyocytomegaly	3/5		1/4
atrophy of germinal testicular epithelium	1/5		
hypocellularity of bone marrow			3/4

- i. Toxicokinetics: Levels were below the LOQ of 2.5 ng/ml for the 10 and 100 µg/kg groups, and for 300 µg/kg after 2 hr.

Toxicokinetic Parameters for 191,305

dose	1000 µg/kg	
	♂	♀
C _{max} (µg/ml)	2.35	2.88
AUC ₀₋₄ (µg·hr/ml)	3.76	4.42
t _{1/2} (hr)	16.2	13.1

73. A single dose intravenous toxicity study of CL 190,396 (N-acetyl-epsilon derivative of calicheamicin), an anticancer agent, in rats (Study 92045). Conducted by [] according to GLP. CL 190,396 is the form of calicheamicin that remains after it has been triggered and inflicted its damage on DNA.

species: CD rats (5/sex/group)

drug: 191,396
 dosage: 0, 10, 100, 300, and 1000 µg/kg NAc-ε-calicheamicin
 age, weight: 7 weeks; 258-320 g ♂, 172-219 g ♀
 observation: 6 weeks
 route: i.v. via tail vein

Observations

- | | |
|--------------------|---|
| Clinical signs | once/daily |
| Body weights | before dosing, twice/wk |
| Food consumption | before dosing, twice/wk |
| Urinalysis | day 0 |
| Hematology | days 13 and 36 |
| Clinical Chemistry | days 13 and 36 |
| Gross pathology | termination |
| Histopathology | termination |
| Toxicokinetics | 0.5, 2, 6, 8, 12, and 24 hr post dosing |
- a. Clinical signs: no mortalities, no treatment related findings
 b. Body weights: no treatment related changes
 c. Food consumption: no treatment related changes
 d. Urinalysis: hemoglobin present due to hemolysis from 4% ethanol carrier
 e. Hematology: no treatment related changes
 f. Clinical Chemistry: no treatment related changes
 g. Gross pathology: 10-20% ↑ relative and absolute heart weight in ♀; 7-33% ↑ relative and absolute thymus weight in ♂ and 1000 µg/kg ♀
 h. Histopathology: no treatment related changes
 i. Toxicokinetics: Toxicokinetics were not calculated because the 190,396 concentrations in plasma fell below the LOQ of 2.5 ng/ml ≤ 2 hr after dosing. C_{max} was linear with dose and in the 1000 µg/kg groups was 90 ng/ml in ♂ and 57 ng/ml in ♀.

B. Multiple Dose Toxicity

63. Six Cycle intravenous toxicity study of CL 555,201 (N-acetyl-gamma-dimethyl-hydrazide-acetyl-butyrloxy derivative of calicheamicin [CL 191,305] conjugated to hP67.6 [CL 555,001]), an anticancer agent, in rats followed by a four week recovery period (Study 94034). Conducted by [] according to GLP.

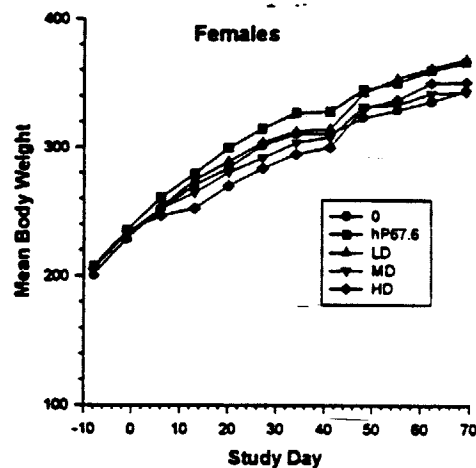
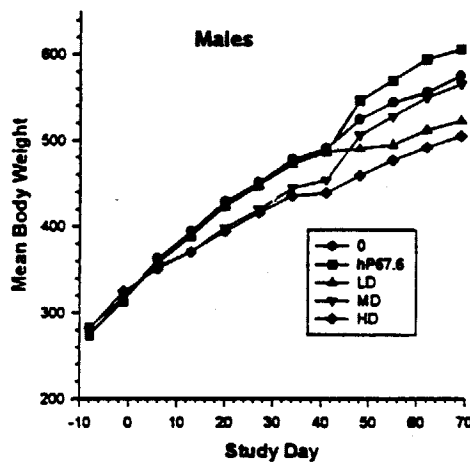
species: CD rats (15/sex/group)
 dosage- hP67.6 equivalents: 0, 0.1, 0.4, and 1.2 mg/kg once/wk x 6 wk plus the naked antibody (555,001) at 1.2 mg/kg
 toxic equivalents: 0, 3, 10, and 30 µg/kg NAc-calicheamicin AcBut (191,305)
 age, weight: 8 weeks; 263-398 g ♂, 203-289 g ♀
 observation: 4 wk
 route: i.v. bolus via tail vein

Observation

Clinical signs	twice/daily; once daily in recovery
Body weights	before dosing, once/wk
Food consumption	before dosing, once/wk
Ophthalmoscopic	before dosing and day 36
Hematology	pre-dose, days 9, 37, and 65
Clinical Chemistry	pre-dose, days 9, 37, and 65
Urinalysis	pre-dose, days 10, 41, and 66

Antibody Monitoring	pre-dose, days 21, 41, and 65
Gross pathology	termination (organs p. 79)
Histopathology	termination
Toxicokinetics	0, 2, 24, 96, and 168 hr post dosing on days 0 and 35

- a. Clinical signs: no mortality; dose related signs were ↓ feces and wet/stained perianal area in ♀s
- b. Body weights: As shown in the following figures, hP67.6 conjugate in ♂ rats caused an 8 and 11% ↓ in MD and HD groups respectively at the end of the dosing period. In ♀ rats, a ~5% ↓ occurred in the HD group on days 13-34 only.



- c. Food consumption: ↓ 12% in MD and HD ♂ week 2; ↓ 17% in MD ♀ week 5 and ↓ 9% in HD ♀ week 1
- d. Ophthalmoscopic: no treatment related findings
- e. Hematology: note that PMN, atypical lymphocytes, and monocyte counts were highly variable during dosing period
- RBC: 10% ↓ in HD ♂, 5% ↓ in naked hP67.6 ♂, 7% ↓ in MD and HD ♀
 - Hgb: 5% ↓ in HD ♂ and MD, HD ♀
 - Ht: 8% ↓ in HD ♂, 6% ↓ in MD, HD ♀
 - normoblasts: ↑ in HD ♀ on day 37
 - MCV: 4% ↓ in HD ♂
 - MCHb: 6% ↓ in HD ♂, 3% ↓ in MD, HD ♀
 - platelets: 18, 23, 29% ↓ in LD, MD, HD ♂ and 19% ↓ in HD ♀ both on day 9
 - WBC: 19, 20, 31% ↓ on day 9 in LD, MD, HD ♂ and 17% ↓ on day 37 in HD ♂
 - lymphocytes: 20% ↓ in LD, MD, HD ♂
- after recovery period
- RBC: 15% ↓ in MD, HD ♀
 - MCV: 13% ↓ in LD ♂, 15% ↓ in HD ♂
 - MCHb: 6% ↓ in HD ♂
 - WBC: 30% ↓ in HD ♀
- f. Clinical Chemistry: small (<5%) changes in Na⁺, Cl⁻, Ca²⁺ were deemed not toxicologically significant
- glucose: 9% ↓ in HD ♂ on day 37; 15% ↓ in MD ♀ on day 9
- mean cholesterol: ♀: 30% ↓ in HD days 9, 37 and 40% ↓ in MD day 37; ♂: 100% ↓ in HD day 37

AST: dose-related 20-45% ↑ in LD, MD, and HD groups days 9 and 37 (♂ more sensitive); normal values after recovery

ALT: dose-related 30-100% ↑ in LD, MD, and HD groups days 9 and 37 (♂ more sensitive); HD ♂ still ↑ 50% after recovery

albumin: ↓ 12% in HD groups on day 37, ↓ 7% in MD ♀ on day 37

globulin: ↑ 15% in MD, HD ♂ days 9,37; ↑ 35% in MD, HD ♀ day 37 and ↑ 14% in HD ♀ day 9

A/G: ↓ 1-25% in MD and HD groups on day 37

total protein: ↓ 7% in MD ♂ days 9,37

alkaline phosphatase: ↓ 16-50% in MD, HD ♂; ↓ 40-140% in HD ♀

triglycerides: ↑ 25% in LD, MD, HD and naked hP67.6 ♂ on day 9

- g. Urinalysis: HD group: ♂: 2+ proteinuria on day 10 and 3+ proteinuria on day 41
♀: 3+ proteinuria on day 41
MD group: ♂: 2+ proteinuria on day 41
LD group: ♂: 2+ proteinuria on day 41

After recovery period, the HD proteinuria did not resolve and 2+ proteinuria was still detected in one LD ♂ and one MD ♀.

- h. Antibody Monitoring: Antibodies to hP67.6 were measured by the ability of ¹²⁵I-hP67.6 to form aggregates with rat plasma as visualized by gel permeation chromatography. Results are shown in the following Table. hP67.6 conjugate was clearly immunogenic in rats.

of Animals With Indicated Levels of Aggregate Formation

	Day 21						Day 41						Day 65					
	-	±	+	++	+++	++++	-	±	+	++	+++	++++	-	±	+	++	+++	++++
PBS	11	9					7	10	3				1	8	1			
naked hP67.6	8	3			6	3	1	2	4		2	11		1	2	2	3	2
LD	2	7			3	8	2	3	1		2	12		2	1		1	6
MD		2	1	1	3	13		2	1		8	8					1	9
HD	4		1	1	6	7		1		1	6	11				1		8

- i. Gross pathology: no treatment related findings in LD, MD, or naked antibody groups;
HD: a mottled red discoloration and rough surface were noted in the liver which resolved in the recovery period; testes appeared small at the end of both dosing and recovery periods; the kidneys were pale at the end of both dosing and recovery periods

Organ weights: As shown in the following table, treatment related changes in absolute and relative organ weights were noted in liver, spleen, kidneys, testes, adrenals, and possibly brain. All weight changes were reversible in 4 wk except in the testes.

Percent Change in Organ Weights

	LD		MD		HD		After Recovery
	Absl	ΔRel	Absl	ΔRel	Absl	ΔRel	
liver ♂	14	12	-	19	42	58	normal

♀	-	-	21	22	24	31	normal
spleen ♂	-	-	-	-	34	47	normal
♀	-	16	-	18	33	41	normal
kidneys ♂	-	-	-	-	14	25	normal
♀	-	-	18	20	-	20	▷LD ♀ ↑15%
testes	-	-	-38	-32	-55	-49	▷HD ↓62%
ovary	-	-	21	23	-	-	normal
adrenals ♂	-	-	-	15	15	28	normal
♀	-	-	-	-	15	23	normal
thyroid	-	-	-	-	-	-	▷hP67.6 & MD ♀ ↓25%
brain ♂	-	-	-	10	-	11	▷hP67.6 ↑10%
♀	-	-	-	-	-	17	(hP67.6 ↓7% in treatment)
pituitary	-	-	-	-	-	17	normal
thymus ♂	29	26	-	-	-	-	normal

j. Histopathology:

mammary gland: minimal to marked atrophy in ♂ at MD and HD that resolved for the MD and moderated for the HD in recovery

kidney: slight to marked tubular dilation, casts, basophilia in HD♂, and MD and HD ♀ which moderated in recover

liver: In HD groups minimal to slight vacuolation, minimal to marked hepatocellular karyocytomegaly (pronounced in ♂), minimal to moderate atrophy of hepatocytes and dilation of sinusoids, minimal to slight oval cell/bile duct proliferation (pronounced in ♀). In the recovery period the vacuolation, atrophy, and dilation of sinusoids improved; the karyocytomegaly, and oval cell/bile duct proliferation worsened. In addition minimal to slight karyocytomegaly of hepatocytes was observed in MD ♂.

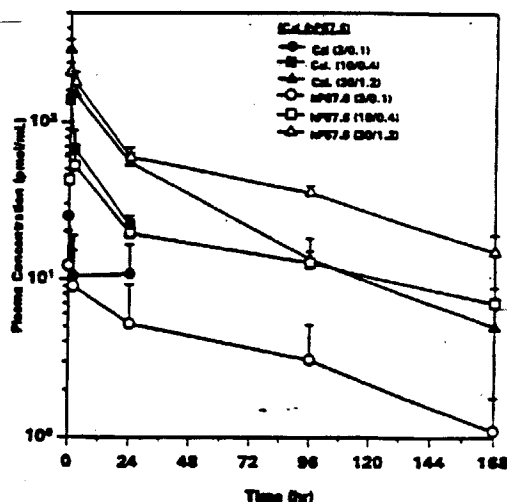
spleen: minimal to moderate atrophy of the marginal zone in MD ♂, HD ♂, and HD ♀. A slight to moderate ↑ in extramedullary hematopoiesis was noted in both sexes in HD groups. All of these changes had resolved by the end of the recovery period.

testes: In HD, slight to marked atrophy of the seminiferous tubules, slight oligospermia, slight desquamated cells in the epididymis, slight hyperplasia of the interstitial cells. The findings did not resolve in recovery.

bone marrow: In HD slight necrosis and/or fibrosis were observed which resolved during recovery.

k. Toxicokinetics: Total calicheamicin (released from conjugate by reduction with mercaptoethanol), free calicheamicin (extracted from plasma with acetone), and total hP67.6 were assessed by ELISA.

The LOQ for calicheamicin was 8.7 ng/ml and for hP67.6 was 10 ng/ml. Insufficient data was collected on Day 35 to arrive at definitive conclusions for many of the parameters. On Day 0, the C_{max} of total calicheamicin and the AUC and C_{max} for total hP67.6 was linear with dose. The toxicokinetic profile on Day 0 is shown in the adjacent figure. No significant differences were noted between ♂ and ♀. Free calicheamicin accounted for 3.6% of the total calicheamicin dose equivalents.



Pharmacokinetic Parameters for hP67.6 Conjugate in Rats

		Calicheamicin			Total hP67.6	
		total		free		
	Dose	Day 0	Day 35	Day 0	Day 0	Day 35
C_{max} (µg/ml)	HD	0.494	0.386	0.0176	31.7	7.97
	MD	0.254	0.182	-	7.7	-
	LD	0.037	0.070	-	1.94	-
AUC_{168} (µg·hr/ml)	HD	8.78	-	-	1180	-
	MD	2.42	-	-	403	-
	LD	-	-	-	91	-
$t_{1/2}$ (hr)	HD	39	-	-	72	-
clearance (ml/hr/kg)	HD	2.9	-	-	0.85	-

64. A six Cycle intravenous toxicity study of CL 555,201 (N-acetyl-gamma-dimethyl-hydrazide-acetyl-butyryloxy derivative of calicheamicin [CL 191,305] conjugate to hP67.6 [CL 555,001]), an anticancer agent, in monkeys followed by a four week recovery period (Study 94001). Conducted by [] according to GLP (except for antibody monitoring report).

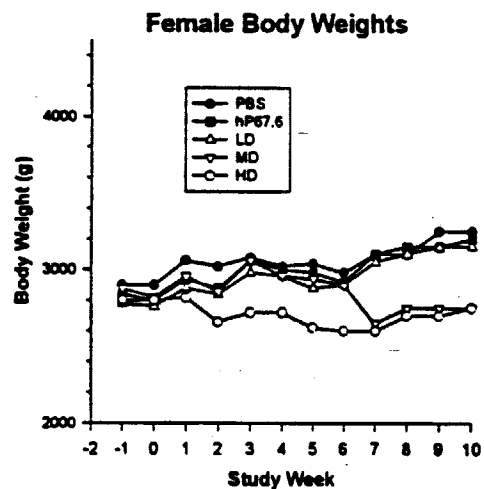
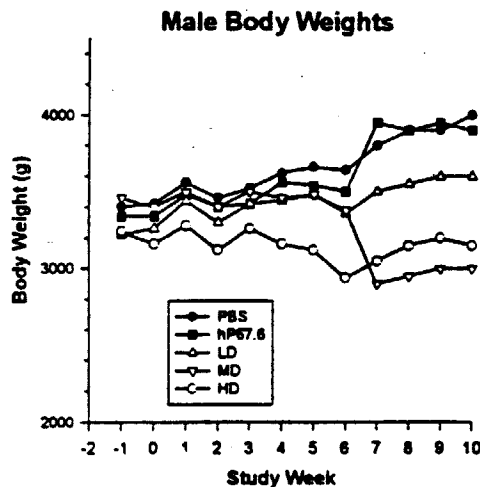
species: monkeys (*Macaca fascicularis*) (5/sex/group)
 drug: hP67.6 conjugate
 dosage- hP67.6 equivalents: 0, 0.2, 0.6, and 1.8 mg/kg once/wk x 6 wk plus the naked antibody (555,001) at 1.8 mg/kg
 toxic equivalents: 0, 5, 15, and 45 µg/kg NAc-calicheamicin AcBut (191,305)
 age; weight: 3-5 yr; 2.5-5.4 kg ♂, 2.4-3.3 kg ♀
 observation: 4 wk
 route: i.v. via saphenous vein

Observations

Clinical signs twice/daily

Body weights	before dosing, once/wk
Appetite evaluation	before dosing, daily
EKG	pre-dose, days 37 or 38 and 69; time not specified
Ophthalmoscopic	pre-dose, day 36
Hematology	pre-dose, days 9, 37, and 65
Clinical Chemistry	pre-dose, days 9, 37, and 65
Urinalysis	pre-dose, days 10, 38, and 66
Antibody Monitoring	pre-dose, days 21, and 65; (non-GLP)
Gross pathology	termination (organs p. 20)
Histopathology	termination
Toxicokinetics	0.5, 2, 6, 12, 24, 48, 72, 120, and 168 hr post dosing on days 0 and 35

- a. Clinical signs: no mortality; no treatment related findings
- b. Body weights: HD caused decreases (~10%) as shown in Figures during entire dosing period; MD caused decreases after 4-6 wk in ♂ and 6 wk in ♀; LD caused a decrease only in ♂ after 4-6 wk



- c. Appetite evaluation: no treatment related findings
- d. EKG: no treatment related findings
- e. Ophthalmoscopic: no treatment related findings
- f. Urinalysis: one HD ♀ had 3+ proteinuria on day 38
- g. Hematology: No treatment related effects in reticulocytes, normoblasts, mean cell hemoglobin concentration (percent), platelets, eosinophils, basophils, monocytes, stabs or atypical lymphocytes. Changes are listed in the following table and appeared to be reversible. Coagulation studies showed minor changes in APTT (<20%!) and PT (<4%!) that were present at days 9 and 37.

Percent Change in Hematology Measurements

	Day 9 (♂, ♀)	Day 37 (♂, ♀)	Day 65 (♂, ♀)
hematocrit	130, 31% HD	145, 46 HD 123, 0 MD	

hemoglobin	131,30 HD	145, 44 HD 123, 15 MD	10, 5 LD
RBC	132, 32 HD	148, 48 HD 131, 0 MD	123, 0 HD 124, 0 MD
MCV		112, 0 MD	119, 0 HD 121, 0 MD
MCHb		113, 0 MD	124, 0 HD 126, 0 MD
WBC	144, 57 HD 134, 28 MD	179, 70 HD 151, 34 MD	159, 40 HD 123, 33 MD
PMN (highly variable predose and control values)		189, 72 HD 10, 14 MD 10, 50 LD 10, 59 hP67.6	
lymphocytes	148, 68 HD 143, 23 MD	167, 70 HD 152, 29 MD	151, 46 HD

h. Clinical Chemistry:

Na⁺ was 12-10% in LD, MD, and HD groups on day 9, 37, and 65;

Cl⁻ was 110% in all groups on day 37;

total protein was 1 ≤ 20% in LD, MD, and HD groups on days 9, 37, and 65;

albumin was 110-20% in LD, MD, and HD groups on days 9, 37, and 65;

globulin was 110-60% in dose related fashion on days 9, 37, and 65;

A/G ratio was 130-50% in LD, MD, and HD groups on days 9, 37, and 65;

BUN and creatinine had a trend of 1 on day 37;

AST was 1 on days 9 (4-fold), 37 (6-fold), and 65 (3-fold) in LD, MD, and HD groups;

ALT was 12.5 fold in HD group on day 9 that was not noted by Sponsor.

None of the above changes were considered biologically significant by the Sponsor, but they suggest to the reviewer that the hP67.6 conjugate was mildly hepatotoxic to the monkeys.

i. Urinalysis: one HD ♀ had 3+ proteinuria on day 38

j. Antibody Monitoring: Antibodies to hP67.6 were measured by the ability of ¹²⁵I-hP67.6 to form aggregates with monkey plasma as visualized by gel permeation chromatography. Results are shown in the following Table. At Day 65 all plasma (2♂ and 2♀) gave negative aggregate formation except 1 naked hP67.6 ♂ had a ++++ response and 1 LD ♀ had a ± response. Samples from day 45 but were accidentally warmed and the assay was thus not conducted.

# of Animals With Indicated Levels of Aggregate Formation at Day 21						
	-	±	+	++	+++	++++
PBS	7	3				
naked hP67.6	6	2		1	1	
LD	3	6		1		
MD	3	5	2			
HD	2	6	2			

Pharmacokinetic Parameters for hP67.6 Conjugate in Monkeys

		Calicheamicin		Total hP67.6
		total	free	
	Dose	Day 0	Day 0	Day 0
C_{max} ($\mu\text{g/ml}$)	HD	1.490	0.0114	3.57
	MD	0.282	-	11.10
	LD	0.111	-	3.50
AUC_{168} ($\mu\text{g}\cdot\text{hr/ml}$)	HD	25.7	-	2530
	MD	5.87	-	795
	LD	2.20	-	258
$t_{1/2}$ (hr)	HD	183	-	176
	MD	115	-	141
	LD	98	-	129
clearance (ml/hr/kg)	HD	1.08	-	0.353

Histopathology Inventory for IND

Study	63	64	68	69	72	73
Species	rat	monkey	rat	dog	rat	rat
Adrenals	X	X	X	X		
Aorta	X	X	X	X		
Bladder	X	X	X	X		
Bone Marrow smear	X	X	X	X		X
Bone (femur)	X	X	X			
Brain	X	X	X	X		
Cecum	X	X	X	X		
Cervix						
Colon	X	X	X	X		
Duodenum	X	X	X	X		
Epididymis	X	X	X	X		
Esophagus	X	X	X	X		
Eye	X	X	X	X		
Fallopian tube						
Gall bladder		X		X		
Gross lesions	X	X	X	X		X
Harderian gland			X			
Heart	X	X	X	X		X
Hypophysis						
Ileum	X	X	X	X		
Injection site	X	X	X	X		X
Jejunum	X	X	X	X		
Kidneys	X	X	X	X		X

Lachrymal gland						
Larynx						
Liver	X	X	X	X		X
Lungs	X	X	X	X		
Lymph nodes, cervical						
Lymph nodes mandibular	X	X	X	X		
Lymph nodes, mesenteric	X	X	X	X		
Mammary Gland	X	X	X	X		
Nasal cavity						
Optic nerves						
Ovaries	X	X	X	X		
Pancreas	X	X	X	X		X
Parathyroid	X	X	X	X		
Peripheral nerve	X	X	X			
Pharynx						
Pituitary	X	X	X	X		X
Prostate	X	X	X	X		
Rectum	X	X	X	X		
Salivary gland	X	X	X	X		
Sciatic nerve						
Seminal vesicles	X	X	X			
Skeletal muscle	X	X	X	X		
Skin	X	X	X	X		
Spinal cord	X	X	X	X		
Spleen	X	X	X	X		X
Sternum	X					
Stomach	X	X	X	X		
Testes	X	X	X	X		X
Thymus	X	X	X	X		X
Thyroid	X	X	X	X		
Tongue	X	X	X	X		
Tonsil		X		X		
Trachea	X	X	X	X		
Ureter	X	X	X			
Urethra	X	X	X			
Uterus	X	X	X	X		
Vagina	X	X	X	X		
Zymbal gland						

C. Special Toxicity

8. The use of radiolabeled anti-CD33 antibody to augment marrow irradiation prior to marrow transplantation for acute myelogenous leukemia. Transplantation. 1992; 54:829-833. Nine AML patients were given 2.0 to 4.0 mg/m² murine P67.6 labeled with ¹³¹I. One patient was given 20 mg/m² which saturated all antigen binding sites in peripheral blood and marrow. 4/9 patients had favorable biodistribution in that the spleen and marrow received higher radiation doses than any normal organ. In the remaining patients, the residence time in the marrow was short probably due to rapid binding, internalization, and release of the iodine. The serum t_{1/2} varied between 0.7 and 31 hr. No dose limiting toxicities were seen. This study demonstrated that antibodies to CD33 can be administered without unexpected tissue binding or adverse toxicity.

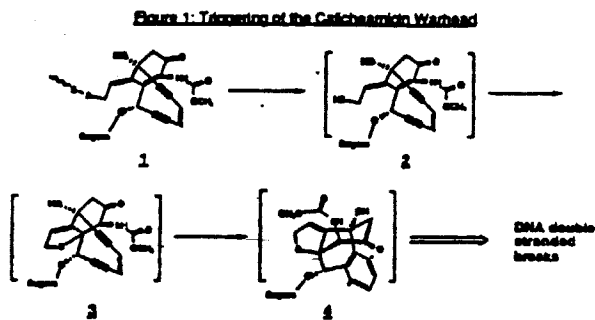
24. Reactivity of humanized hP67.6 conjugate (CPP771/CL555201) to a panel of normal human tissues. Conducted by [] according to GLP on frozen tissues obtained from []
[] hP67.6 conjugate reactivity at 10 µg/ml was tested on 50 tissues from 51 individuals.

Twenty tissues were tested from 3 separate sources, 9 tissues were tested from duplicate sources, and the remaining 21 tissues were tested from single sources. Binding of the conjugate was detected with a rabbit anti-idiotypic anti-hP67.6 followed by donkey anti-rabbit antibody linked to horseradish peroxidase. HL-60 smears were the positive control and adjacent sections without the hP67.6 conjugate served as the negative control. The reactivity pattern was similar to the biotinylated hP67.6 (see studies 56, 57). Specific staining was observed in 26 out of 99 sections which accounted for 18 out of 50 tissues. Apparent staining of endothelial cells was attributed to tissue histiocytes. Reactivity by grade was as follows:

borderline: spinal cord, striated muscle
 weak: adrenal, cerebellum, cervix, colon, lung, lymph node, pancreas, parathyroid, pituitary, spinal cord, spleen, stomach, thymus, gall bladder, bone marrow
 moderate: spleen, ileum

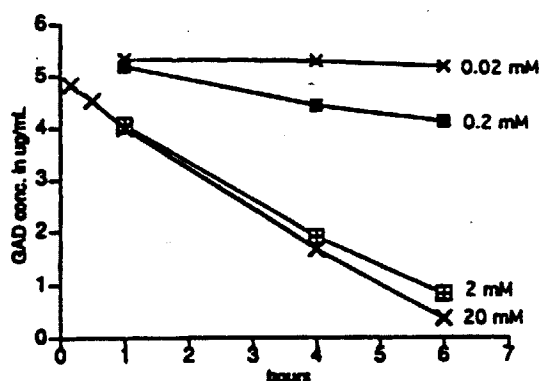
This reactivity was attributed to cells of macrophage/monocyte lineage by staining with CD68. No adverse reactivities were thus observed.

48. **Reaction of N-Ac calicheamicin DMH (CL 184,538) with reduced glutathione.** Conducted by [] Reduction of the disulfide bond in the conjugate triggers the calicheamicin residue for rearrangement as shown in the following diagram (Vol 1.10, p. 130).



The reaction of 184,538 with glutathione in 20% ethanol in PBS, pH 7.4 with 1 mM EDTA at 37°C was monitored by [] Numerical data on $t_{1/2}$ or rate constants was not provided; the disappearance of starting material is shown in the following figure (Vol 1.10, p. 134). In conclusion, 184,538 was stable to "triggering" at glutathione concentrations found in plasma ($<20 \mu\text{M}$), but was reactive with typical intracellular levels of glutathione (2 mM).

Figure 6: Reaction of N-Ac Gamma DMH with Glutathione. Experiment 2



54. **Hydrolysis of hP67.6-N-Ac gamma calicheamicin DMH AcEt conjugate (CL 555,201) in buffer at physiologically relevant pH's.** Conducted by [] Release of calicheamicin from the conjugate can occur either through hydrolysis of the hydrazone or disulfide exchange. The hydrolysis of the hP67.6 conjugate (0.5 mg protein/l) was monitored by the release of NAc-

calicheamicin DMH by [] (225 nm). The buffer was 27.5 mM Na phosphate, 100 mM NaCl, 50 mM sucrose adjusted to pH 4.5, 6.0, or 7.4. At pH 7.4, 2-3% of the calicheamicin was released every 24 hr. At pH 4.5, which occurs in lysosomes, half of the maximal release occurs by 10 hr. Only about half of the total expected calicheamicin appeared to be released, but data was shown that significant amounts of the released drug was bound to glass.

56. Reactivity of humanized P67 antibody (CDP771) to normal human tissues. Conducted by [] according to GLP with samples obtained from [] Biotinylated hP67.6 applied at 10 µg/ml had negative staining in 15 of 44 tissues samples from 30 individuals. In 10 tissues reactivity with leukocytes was observed. In 15 tissues staining of blood vessel "endometrial" cells was observed and 11 of these tissues also had leukocyte staining. The monocyte smear and ovarian luteal cells stained positive. The blood smear and bone marrow preparations had degraded and could not be evaluated. Positive staining was cytoplasmic in all cases. Since the positive staining was unexpected, representative positive tissues were reanalyzed with an additional blocking step, but similar results were obtained. Staining without the primary antibody was negative in all tissues. Staining was negative in striated muscle, and positive in HL-60 cells at the cell membrane. It is possible that fixation exposed a cytoplasmic immunoreactive epitope.

18. Reactivity of humanized antibody (CDP771/CL555001) to a panel of normal human tissues. Conducted by [] according to GLP on frozen tissues obtained from [] This study was an extension of study #56 to increase the number of specimens and to identify better the reactive cell types. When results were combined with study #56, biotinylated hP67.6 reactivity at 10 µg/ml was tested on 47 tissues from 47 individuals. Nineteen tissues were tested from 3 separate sources, 7 tissues were tested from duplicate sources, and the remaining 21 tissues were tested from single sources. Binding of the conjugate was detected using a biotin/avidin detection system. HL-60 smears were the positive control and adjacent sections without the biotinylated hP67.6 served as the negative control. Specific staining was observed in 62 out of 94 sections which accounted for 36 out of 47 tissues. This reactivity was attributed to cells of macrophage/monocyte lineage by staining with CD68. Apparent staining of endothelial cells was attributed to tissue histiocytes. Co-incubation with CD33 abolished specific staining. Tissue reactivity patterns were shared with another CD33 antibody, MY9. Reactivity was confirmed to be specific for CD33 antigen and not due to specific cell binding of the human IgG4 isotype. No adverse reactivities were thus observed with human tissues.

57. Cross reactivity of humanized monoclonal antibody hP67.6 with tissues of cynomolgus and Sprague-Dawley rats. Conducted by [] according to GLP. Biotinylated hP67.6 was applied to monkey and rat tissues at dilutions of 1:50 and 1:500. Strong staining of some tissues was noted but was considered negative on the basis that either: a) labeling was cytoplasmic rather than on the plasma membrane, b) other sections of the same sample were negative, c) positive staining was also noted with the control antibody, biotinylated humanized IgG4.

59. Cardiovascular safety assessment of CL 555,201 in conscious beagle dogs, study 94C. A non-GLP study conducted by [] as three separate studies.

species:	beagle dogs (2/sex/group)
drug:	hP67.6 conjugate
dosage- hP67.6 equivalents:	4, 13, 40 mg/m ² (compared to vehicle in same animal on previous day)
toxic equivalents:	100, 330, 1000 µg/m ² NAc-calicheamicin AcBut (191,305)
age; weight:	10-26 mo; 10.5-13.3 kg
observation:	~30 min prior to ~60 min post dosing utilizing the CPMS
route:	i.v. 30 min infusion except 40 mg/m ² was bolus

dose	mean arterial pressure	heart rate	cardiac output	ECG
4 mg/m ²	5%↓ at 55, 85 min	-	10%↓ at 50 min	-
13	↓ trend (10%) 25-60 min	40% ↑ at all times ≥ 15 min	-	P-wave amplitude ↑ 30% at 20-60 min; T-wave amplitudes ↑ 45-140% at all times ≥ 15 min
40	~20%↓ at 5-25 min	rising trend 15-60 min	40%↓ 5-10 min	P-wave ↑ trend at 5 min w/ sign. at 40 min only (25%); 160% ↑ T-wave 5-45 min

66. In vitro blood compatibility of CL 555,201 (N-acetyl-gamma-dimethyl-hydrazide-acetyl-butyryloxy derivative of calicheamicin [CL 191,305] conjugated to hP67.6 [CL 555,001]), an anticancer agent. (Study 94052). Conducted by [] according to GLP except that the dosing solution analysis lacked appropriate documentation. hP67.6 conjugate was added to whole human blood diluted 1:10 in saline to give 0.3 µg/ml which is 10-fold higher than expected in humans. The tubes were incubated at room temperature with occasional mixing for 30 min and then centrifuged and inspected visually for hemolysis (pellet formation and plasma color). Saline and water alone were used as negative and positive controls. Samples were also inspected for protein flocculation after 10 min. No evidence of hemolysis or protein flocculation was found.

86. The effects of the derivatives of gamma calicheamicin, N-acetyl gamma calicheamicin (CL 181,927), N-acetyl dimethyl hydrazide (CL 184,538), N-acetyl dimethyl acid (CL 186,760) and N-acetyl epsilon (CL 190,396) on murine bone marrow hematopoietic colony formation in vitro (Study 92166). Conducted by [] to assess myelotoxic potential and lineage specificity. Mouse bone marrow cells were added to soft agar containing various growth factors and calicheamicin analogues. Concentration dependent myelotoxicity was observed for all compounds except N-Ac-ε-calicheamicin which was not toxic below 10 nM; no lineage specificity was apparent with any of the compounds.

IC₅₀ (nM) for Inhibition of Colony Formation

Colony type	N-Ac-γ-cal'	N-Ac-cal' DMAcid	N-Ac-cal' DMH	N-Ac-ε-cal'
CFU-E	1.8	1.3	2.7	>10
CFU-GM	2.9	3.5	3.5	>10
BFU-E	2.4	1.3	3.6	>10
CFU-GEMM	2.3	1.4	5.7	>10

93. In vitro stability of hP67.6 conjugate (CL 555,201) in human, monkey, and rat plasma at 37°C for a period of 6 hours. A non-GLP study conducted by [] hP67.6 conjugate at 100 µg/ml was incubated with human, monkey, and rat plasma. Duplicate 0.1 ml samples at 0, 0.5, 1, 2, 4, and 6 hr were analyzed by ELISA for unconjugated calicheamicin and at 0 and 46 hr for total calicheamicin. A standard sample of 184,538 at 5 µg/ml was analyzed in parallel. As the following table (next page) shows, hP67.6 conjugate was very stable in plasma.

Unconjugated Calicheamicin as Percent of Total

time (hr)	0	0.5	1	2	4	6
human	1.9	2.0	2.6	2.6	2.9	4.5
monkey	2.4	2.6	3.3	3.3	4.9	6.8
rat	2.3	3.2	3.1	2.9	4.3	4.9

SUMMARY OF TOXICOLOGY DATA**Summary of single dose toxicity**

Single dose studies were conducted in rats, monkeys, and chimpanzees. The LD_{10} is between 8.4 and 12 mg/m² in rats, and between 36 and 54 mg/m² in monkeys. At double the planned human starting dose, 0.5 mg/m², hP67.6 conjugate induced no toxicologic effects in chimpanzees except 2-3 fold elevations in ALT and AST and an 80% increase in WBC. In rats and monkeys the primary toxicities were predominantly to the liver, kidney, spleen, lungs, and GI tract.

The toxicity data for various free calicheamicin derivatives is summarized as follows.

Compound	species	LD_{10}		target toxicities
		µg/kg	µg/m ²	
184,538 NAc-calicheamicin DMH	mice	>500	>1500	hepato
184,538 NAc-calicheamicin DMH	rat	100<300	600<1800	hepato, nephro, myelo, gastric
164,538 NAc-calicheamicin DMH	dog	dose which when doubled did not kill the dogs was 250 µg/m ² 12.5 µg/kg		nephro, myelo, GI,
191,305 NAc-calicheamicin DMH AcBut	rat	100<300	600<1800	hepato, nephro, myelo, gastric
190,396 NAc-ε-calicheamicin	rat	>1000	>6000	-

Summary of multiple dose toxicity

Humans in the proposed trial will receive up to 3 weekly doses of hP67.6 conjugate. Rats and monkeys were administered 6 weekly doses and observed for an additional 4 weeks. The findings summarized in the following table indicate that hP67.6 conjugate at high doses is hepatotoxic, nephrotoxic, and myelotoxic.

Major Toxicologic Disturbances by Category Induced by Multiple Doses of hP67.6 Conjugate

	HD mg/m ²	Clinical Chemistry	Hematology	Gross Pathology	Histopathology
rat	7.2 x 6	cholesterol, AST, ALT, AP, albumin, globulin, A/G, total protein, triglycerides, glucose, proteinuria	RBC, WBC, lymphocytes, platelets	discolored/rough liver, pale kidneys, small testes	liver- atrophy, karyocytomegaly, vacuolation, oval cell/bile duct proliferation; kidney- tubular dilation, basophilia; testes- atrophy
monkey	21.6 x 6	AAT, ALT, albumin, globulin, A/G, total protein, BUN, creatinine, proteinuria	RBC, Ht, Hg, MCV, MCHb, WBC, lymphocytes	red liver foci, small thymus, gelatinous bone marrow	liver- atrophy, pigmented Kupffer cells, dilation of sinusoids, single cell necrosis; kidney- tubular dilation, basophilia, casts, eosinophilic material, vacuolation; lymphoreticular- lymphocyte depletion in thymus, spleen, lymph nodes, and tonsils; hypocellular marrow